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PLANT AND SOIL

International Journal of Plant Nutrition, Plant Chemistry, Soil Microbiology and Soil-borne Plant Diseases

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PLANT AND SOIL

International Journal of Plant Nutrition, Plant Chemistry, Soil Microbiology and Soil-borne Plant Diseases.

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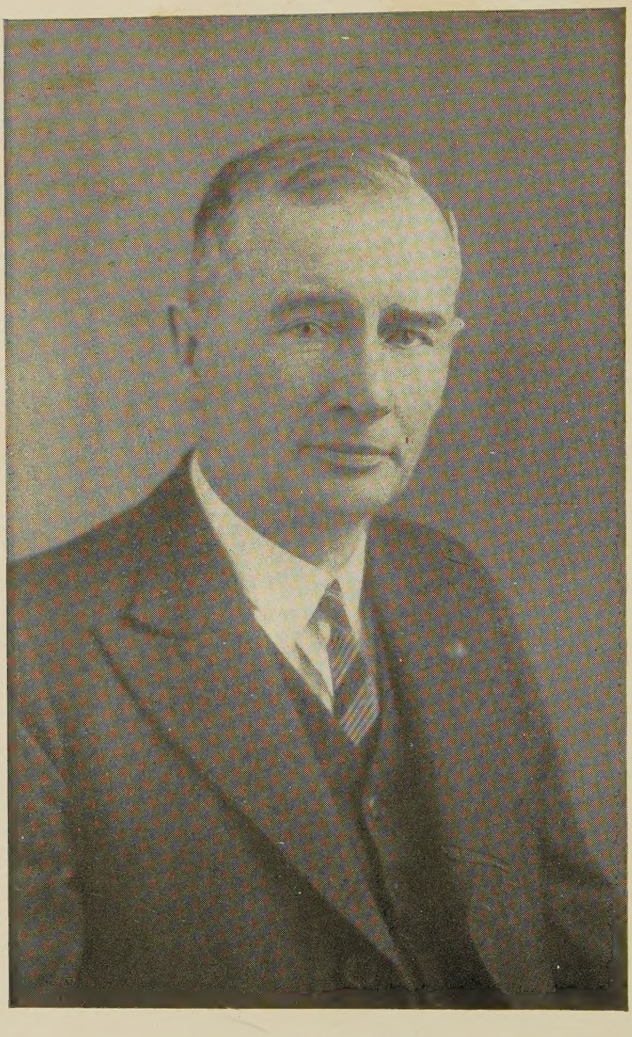

Prof. Dr. O. DE VRIES

PROF. DR. O. DE VRIES †

Professor Dr. O. de Vries who was one of the consulting editors of „Plant and Soil" died on November 27, 1948, following a prolonged illness.

Otto de Vries was born on July 2, 1881, at Amsterdam, where his father, Hugo de Vries, was professor of botany. He studied chemistry at the University of Leiden, presenting the thesis for his doctorate there in 1908. The following year he left Holland for Java to become a chemist at the Tobacco Experimental Station at Klaten. In 1915 he was appointed director of the newly founded Rubber Experimental Station at Buitenzorg, and in 1929 became professor of chemistry at the Medical University of Batavia.

In 1930 he returned to Holland where he took charge of the Agricultural Experiment Station and the Soil Testing Laboratory at Groningen. After Hissinks' retirement in 1939 the Institute for Soil Research also came under his direction.

When in 1943 the Departments for Agricultural Research and for Agricultural Industries of the Central National Council for Applied Scientific Research in the Netherlands (T.N.O.) were founded, de Vries was appointed chairman of both. He left Groningen in 1944 and lived at the Hague until his death.

Professor de Vries was one of the leading authorities in the Netherlands on soil science and application of fertilizers. He represented his country as a delegate to many scientific congresses and meetings.

During his stay in the tropics he undertook a great many investigations into problems of tobacco and particularly rubber. More than a hundred publications covering this period were listed in „Chemisch Weekblad 26, 92–94 (1929)". For his investigations on rubber he was awarded the Colwijn Gold Medal.

At Groningen de Vries was mainly occupied in organizing scientific research at the agricultural institutes under his direction.

Nevertheless he found time to prepare a number of important articles, mainly on soil and soil fertility.

When shortly after the war plans were made to found an international journal of plant nutrition etc., de Vries gave many valuable suggestions and detailed advice as to how to carry them into effect. It was he who proposed the name „Plant and Soil" for the new journal.

E. G. MULDER.

Plant and Soil I, no 3 January 1949

A RAPID METHOD FOR OBTAINING READILY SOLUBLE PHOSPHATES AND PHOSPHATE FIXATION IN SOILS

by B. E. BEATER

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In this paper an attempt has been made to present a rapid and effective technique for assessing the phosphate status of the soil. It claims the advantage of simplicity and ease of manipulation, and is therefore well adaptable to field laboratories.

Reagents and Standards

Extracting Solution: Prepare 2 litres of N/15 H_2SO_4. To this add 1 g pure sodium borate, and dissolve. This buffered solution, origanally used, was found too strong and subsequently diluted to N/21 by making 720 ml to 1 litre. The pH value of the latter solution, now used, is 1.5.

Ammonium Molybdate: Dissolve 25 g of pure ammonium molybdate in 250 ml water. To this add 750 ml of 50 per cent (by volume) H_2SO_4. Keep stored away from direct light.

Stannous Chloride: Dissolve 0.1 g tinfoil in 2 ml conc. HCl by warming. Make up to 10 ml with water. This operation is conveniently made in a test tube with the 2 ml and 10 ml levels scratched on the side. The solution if stoppered will last several days and need not be preserved under liquid paraffin.

Standard Phosphate Solution: Dissolve 1.920 g potassium dihydrogen phosphate in 1 litre of water. Take 10 ml of this stock solution and mix it together with 720 ml of the above undiluted extracting solution in a litre flask. Make to mark with water and mix. 25 ml will now contain 200 lbs P_2O_5 per acre of 2,000,000 lbs soil.

*) Publication authorised by the Director.

Colour Standards: In the analytical procedure below, 2.5 g soil are shaken with 25 ml extracting solution, hence 2 ml extract, the quantity taken for the test, represent 0.2 g soil. If we now take a 0.001 per cent P_2O_5 solution by dissolving 0.0192 g potassium dihydrogen phosphate in a litre (actually, dilute the standard), then 2 ml contain 0.00002 g P_2O_5. If the colour developed in these 2 ml of standard matches that in the 2 ml soil extract when developed, (i.e. in 0.2 g soil), then the soil contains, by simple calculation, 200 lbs P_2O_5 per acre. From this the following table can be arrived at: —

KH_2PO_4 solution	Equivalent to
2.00 ml	200 lbs P_2O_5 per acre
1.50	150
1.00	100
0.80	80
0.50	50
0.40	40
0.30	30
0.20	20
0.10	10
0.05	5

The procedure for developing the colours is the same as for the ordinary test, below, making up to 7 ml in the tube.

Artificial colour standards to match the above have now to be prepared.

For this purpose, dissolve 5 g nickel ammonium sulphate in 70 ml water in a 100 ml flask. Now add 20 ml 5N ammonium hydroxide and mix well. Add 10 g ammonium sulphate, and shake to dissolve. Complete to 100 ml mark, shake, and filter if necessary. It was found that ammonium sulphate was necessary to prevent hydrolysis taking place when the solution was later greatly diluted. Above the range 50 lbs P_2O_5 per acre, the colour of the ordinary phosphate solutions was a less pure blue than that produced by the above solution. The addition of a few drops of 0.2 per cent potassium dichromate was found to remedy this. The quantity of ammonia added affects the final colour, a purplish tinge resulting if a larger quantity than that recommended is added. The following dilutions were found by experiment:

Nickel sulphate solution	0.2 per cent pot. dichromate	Water	Equivalent to P_2O_5 per acre
14.2 ml	7 drops	10 ml	200 lbs
10.5	5	10	150
6.2	4	10	100
4.8	2	10	80
2.5	Nil	10	50
2.1	Nil	10	40
1.5	Nil	10	30
1.2	Nil	10	20
0.7	Nil	10	10
0.4	Nil	10	5

In preparing these artificial standards, the water is placed in a small beaker and the blue solution fun into it from a burette. The tubes (approx. $\frac{3}{8}$ in. internal diam. $\times$ 6 in.) on completion are tightly stoppered or sealed, and are servicable for a year or longer.

Failing the preparation of these colour standards, a series of four to six colour standards may be developed for each dozen soils tested. A suitable colour series would be 20, 40, 60, 80, 100 and 150 lbs P_2O_5 per acre, the P_2O_5 solutions being kept in stoppered bottles and so prepared that 2 ml taken from each would represent the above quantities when developed.

Procedure and Discussion

2.5 g of air-dry 1 mm mesh soil are used. This may be taken up with a graduated spoon if a large number of samples is to be examined. The solution is shaken with 25 ml extracting solution in a 175 ml conical flask, the temperature of the bulk extracting solution being maintained in the neighbourhood of 30°C. This is of great importance, as it was found, for example, that a soil containing 48 lbs P_2O_5 per acre at 27°C, contained 40 lbs at 17°C and only 16 lbs at 7°C.

The shaking time is 30 seconds, after which the contents are poured into filters of the approximate specification of Whatman No. 41, and the whole of the liquid, after returning the first turbid runnings, is allowed to pass through the filter before mixing and using.

2 ml lots are then drawn off and run into test tubes of internal diameter $\frac{3}{8}$ in. and about 6 in. long, with a mark indicating the 7 ml volume scratched on the side. It is advisable to prepare two dozen such tubes in a rack. To the 2 ml now add 3 drops of ammonium molybdate reagent, make nearly to 7 ml mark with water, invert

over clean finger, add 3 drops stannous chloride solution, then invert again twice. Compare with standards after about four minutes. Should the soil be very high in phosphate, take 1 ml extract, or even less.

In light sandy soils, the pH value of the extracting solution is almost unaffected by the soil. In a heavy, moderately alkaline soil, the pH value of the extracting solution may rise a little to 1.7. The pH value of the extract, however, might profitably be checked in the case of calcareous soils, none of which come within the writer's experience.

The above soil to extracting solution ratio of 1 : 10 should be suitable for most normal soils.

Having made the extraction, it now remains to determine the power of the soil to retain phosphate.

For this purpose shake 2.5 g of the soil used for extracting phosphate with 25 ml of the phosphate "fixing" solution, that is, the extracting solution enriched with a known quantity of phosphate. Repeat the procedure exactly as for the ordinary extraction, comparing with the same colour standards. The results may now be set out as in the following examples:

Sample No.	P_2O_5 extracted per acre	P_2O_5 not fixed p. a.	P_2O_5 fixed p. a.	P_2O_5 per cent fixed
1	35 lbs	160 lbs	75 lbs	38
2	Trace	150	50	25
3	55	190	65	33
4	10	80	130	65

Regarding the effect of time on fixation under the conditions of the test, no appreciable differences were found in fixation over 30 seconds or 30 minutes, using a number of soils. A period of 42 hours, however, revealed a further considerable fixation in clayey soils, but only a negligible amount in sandy soils, as would be expected. In view of the fact that fixation in the field does not take place under the ideal conditions of the test, the standardised procedure of shaking for 30 seconds, (which has no disadvantage over shaking up to half an hour or so) was thought to be a better guide to actual field fixation.

Fixation in the presence of a solvent is obviously better than fixation in plain water solution, as phosphates which can be removed by

the solvent action of plant roots cannot correctly be regarded as fixed.

Assessment of phosphate status

A highly significant correlation coefficient (0.8182) was obtained for P_2O_5 analyses by the rapid and one per cent citric acid methods respectively. The arithmetical average of 44 tests, taken at random was 81.4 lbs P_2O_5 per acre by the rapid technique and 119.0 lbs per acre by the one per cent citric acid method, or expressed as percentages, 0.004 per cent P_2O_5 and 0.006 per cent P_2O_5 respectively.

There are many scores of one per cent citric soluble phosphoric acid figures available in the records of this laboratory, together with a general knowledge of the field performance of the soil. From this data it was concluded that soils containing more than 0.020 per cent citric soluble P_2O_5 would not give responses to crops, other things being equal, and were in the "very high" range. Percentages less than 0.001 per cent citric soluble P_2O_5 were likewise observed to be highly deficient in this element, and classed in the "very low" range. The whole range produced is given as follows:

Very low	0—0.001 per cent P_2O_5 (0—20 lbs P_2O_5 p. a.)
Low	0.001—0.003 per cent P_2O_5 (20—60 lbs P_2O_5 p. a.)
Medium	0.003—0.010 per cent P_2O_5 (60—200 lbs P_2O_5 p. a.)
High	0.010—0.020 per cent P_2O_5 (200—400 lbs P_2O_5 p. a.)
Very high	Above 0.020 per cent P_2O_5 (Above 400 lbs P_2O_5 p. a.

Since the mean conversion factor for the citric soluble figures to the writer's rapid results was $\frac{2}{3}$, the above range would, when given to the nearest round number, become:

Very low (*VL*)	0— 10 lbs P_2O_5 per acre.
Low (*L*)	10— 40 lbs P_2O_5 per acre.
Medium (*M*)	40—120 lbs P_2O_5 per acre.
High (*H*)	120—240 lbs P_2O_5 per acre.
Very high (*VH*)	Above 240 lbs P_2O_5 per acre.

These ranges and symbols should serve as a useful guide in assessing the so-called available phosphate status of the soil in its relation to the plant (in this case sugar cane).

With regard to the ranges and symbols for phosphate fixation, the following is offered as a provisional guide:

Very low rate (*VL*)	0— 25	lbs P_2O_5 fixed per acre.
Low rate (*L*)	25— 50	lbs P_2O_5 fixed per acre.
Medium rate (*M*)	50—100	lbs P_2O_5 fixed per acre.
High rate (*H*)	100—150	lbs P_2O_5 fixed per acre.
Very high rate (*VH*)	Above 150	lbs P_2O_5 fixed per acre.

Summary

Following well known methods for the preparation of reagents for the determination of phosphates by the coeruleo-molybdate reaction, the preparation of a new series of colour standards is detailed.

The procedure for estimating phosphate by a rapid technique is given and discussed, important points being ratio of extracting solution to soil, time of reaction and temperature of reaction. The development of the blue colour is otherwise carried out in the recognised manner.

After the extraction of phosphates from the soil, the amount fixed from a phosphate solution in the presence of the same extracting solution by a further sample of the soil of the same weight is next determined, using the same technique. From these two results, the readily soluble phosphate in the soil and the fixation rate are arrived at.

Tentative ranges and symbols are given for readily soluble phosphate in lbs per acre, and also for the fixation rate in lbs per acre.

Received July 23, 1948.

Plant and Soil I, no 3 January 1949

THE SOIL PERCOLATION TECHNIQUE

by H. LEES

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Introduction. The soil percolation technique for the study of the metabolism of whole soil was first described in 1944 [1]). In this technique small quantities of a water solution of metabolites are taken frequently and repeatedly from a bulk of the solution contained in a reservoir vessel, percolated (together with an adequate air supply) through a column of sieved soil, and returned to the bulk of solution in the reservoir. Metabolic processes occurring in the soil may then be followed by analyses of the reservoir fluid. At any time fresh metabolite may be added to the reservoir fluid whence it will be quickly distributed throughout the soil, or conversely the soil processes may be almost instantly selectivily poisoned by the addition of a suitable compound to the reservoir fluid; the reservoir fluid may be sampled as often as necessary without the soil's being disturbed. In fact the technique makes the study of soil metabolism as convenient as the tissue perfusion technique has made the study of animal-tissue metabolism. Initially the soil percolation technique was analogously called the 'soil perfusion technique'. I am indebted to Dr. N. W. Pirie of Rothamsted Experimental Station, England, for pointing out that in this context 'perfusion' is a catachresis and for suggesting the correct 'percolation'.

The original technique was based on a soil percolator designed by myself. Since that time this percolator has been continually modified until it is now a simple, self-regulating device constructed from standardized interchangeable parts. Although the percolator in its final form has been described [2]), ancillary equipment for its operation has not, nor has there been any recent survey of the potentialities or drawbacks of the percolation technique as a whole. This paper is an attempt to make good these deficits.

Equipment. 1. The soil percolator. This basic apparatus [2]) is shown diagramatically for reference purposes in Figure 1. It will function satisfactorily over wide ranges of soil-weight or reservoir-volume.

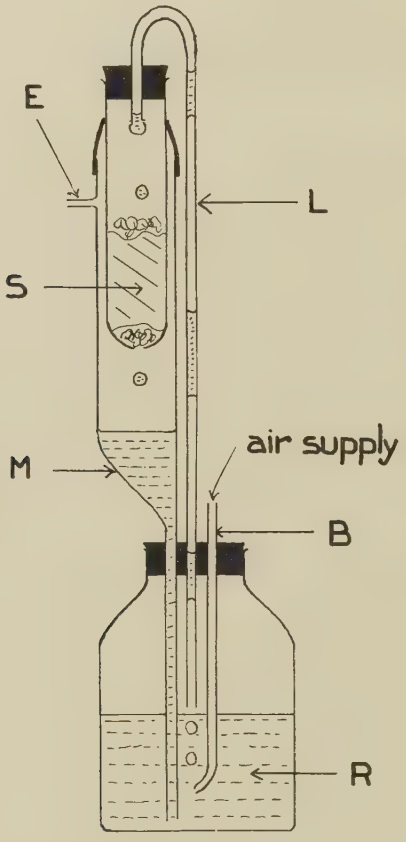

Figure 1. The soil percolator.

Air is forced into the bubbler tube *B* and, bubbling through the reservoir liquid held in *R*, escapes to the atmosphere through the exit tube *E via* the lift tube *L* and the soil in the soil-tube *S*. In so doing it carries with it bubbles of reservoir liquid which pass through the soil and return to the bulk of the reservoir liquid by dripping into the main tube *M*. During percolation there stands in *M* a head of reservoir liquid equivalent to the pressure drop between the surface of the reservoir liquid and the exit of *S* (effectively, the pressure drop across the soil column). Should the soil column be-

come difficulty pervious the head in M rises, the level in R consequently falls, and percolation of the soil ceases because the height of the mouth of L above the level in R becomes so great that no bubbles of liquid can be projected into L. The soil cannot therefore become waterlogged.

The percolator as originally described [2] was driven by suction applied to E, the actuating air was therefore taken through B from the atmosphere. The change to an air-pressure drive applied to B has been made because:

a) The air-supply to any number of percolators may be wetted, scrubbed free of contaminant gases, and sterilized, at source.

b) The air-pressure in the percolator is above that of the atmosphere. Therefore all air movement tends to be outward from the interior of the percolator, and contamination of the soil or reservoir fluid by air-borne organisms is minimized.

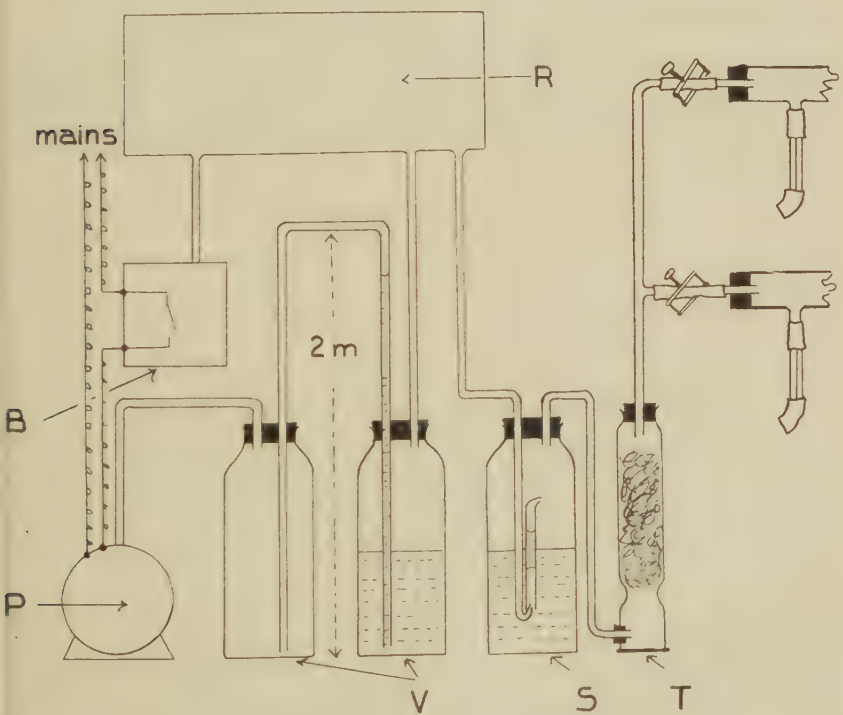

Fig. 2. The air supplying system.

2. The air-supplying system. The layout of this is shown in figure 2.

An electric pump *P*, switched off and on by the barostat relay *B*, intermittently supplies air to a reservoir *R*, maintaining therein an air pressure of between 0.5 metres of water (at which pressure *B* switches *P* on) and 1.5 metres of water (at which pressure *B* switches *P* off). Between *P* and *R* is a non-return water valve *V*, constructed of two Winchester bottles connected by an inverted '*U*' of glass tubing rising to a height of 2 metres, that prevents a backward escape of air from *R* through *P* when *P* is switched off.

From *R* the air proceeds through a scrubbing bottle *S* to a tower *T* filled with fine glass wool to trap any spray of scrubbing liquid and thence, through lengths of rubber tubing controlled by screw clips, to metal pipes of 2 cm internal diameter each feeding 12 percolators. These pipes, roughly 1.4 metres long, carry at 12 cm intervals metal side arms to each of which is attached an 8 cm length of glass capillary tubing (0.5 mm internal diameter) permanently carrying on its distal end a short length of rubber pressure tubing to which a percolator may be connected as required.

If the screw clip controlling each "bank" of 12 percolators is adjusted so that the air through-put of each percolator is about 1–2 ml air/sec., the air turbulence at the constriction applied by the screw clip tends to limit the maximum air flow to the bank and the percolators function fairly steadily over the 1 metre pressure variation in *R*. Because the side-arm capillaries act similarly as air-flow limiters, one percolator will run satisfactorily when the other 11 positions on the "bank" are empty and are thus bleeding the air supply at maximum rate. It is therefore never necessary to cut off a bank completely in order to change any number of percolators on it.

The various units comprising the assembly just described will now be considered in detail.

2*a*. The pump. A rotary centrifugal pump is the most satisfactory type, small diaphragm 'aerators' are not suitable for long-term operation because the rubber valves tend to perish. Whatever the pump it should be capable of supplying air at least ten times as rapidly as the percolators consume it so that for 90% of the operating time the pump is switched off and therefore remains reasonably cool.

2*b*. The reservoir. This should provide at least 10 litres storage capacity per percolator. At the recommended through-put of 1—2 ml air/percolator/second and at the recommended reservoir-pressure range of approximately 0.03 atmospheres, such a capacity enables the percolators to run for $2^1/_2$–5 minutes without assistance from the pump, the motor of which therefore has some chance to cool between operating periods. The assembly of 24 percolators shown in Plate 1 is operated from a reservoir consisting of a discarded oil drum of about 250 litres capacity.

2*c*. The barostat relay. This is a single-pole single-throw switch included in one lead of the mains supply to the motor of the pump and operated by the air pressure in *R*. When the pressure in *R* drops to 0.5 metres of water the pump is switched on, and when the pressure has been raised to 1.5 metres the pump is switched

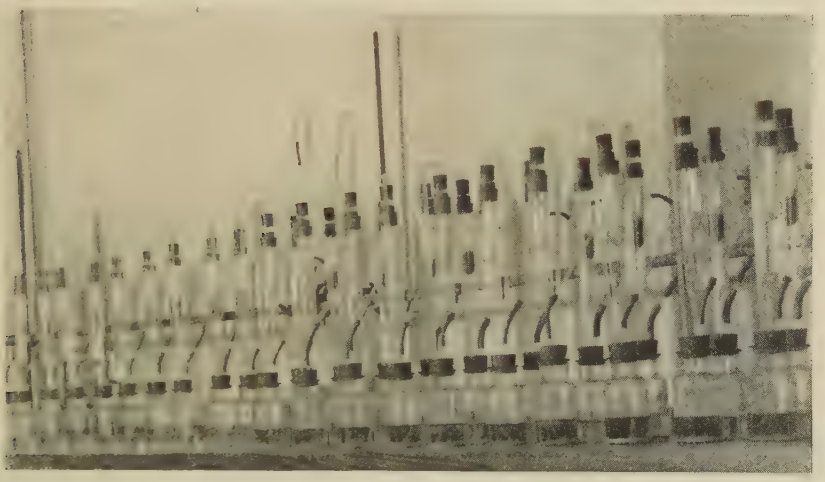

Plate 1. Percolator assembly. The nearer bank is being used for carbon dioxide measurements.

off. The relatively large 1 metre delay reduces the switching-frequency of the pump motor and therefore reduces wear on its starter brushes.

Of the many types tested, the simplest and most reliable relay has been found to be the 'tipping relay' shown in Figure 3. At all increasing reservoir pressures below breaking pressure (1.5 m water) the mercury manometer assembly remains level and contact *C*, which is the switch controlling the pump motor, remains closed. Breaking pressure is reached when, as a result of the rising pressure in the air reservoir *R*, enough mercury has been forced from the L.H.S. of the manometer to the R.H.S. to cause the whole assembly to tip up onto stop *S*, break contact *C*, and thus switch off the pump motor.

Because the assembly is suspended above the fulcrum, the effect of tipping is to shift the C. of G. of the L.H.S. of the assembly horizontally nearer to the fulcrum and concomitantly to shift the C. of G. of the R.H.S. further away. At the same time mercury runs from the L.H.S. to the R.H.S. in order to preserve the same vertical difference in height between the head of mercury in the L.H.S. and that in the R.H.S. On both these counts, as soon as breaking pressure is reached and without there being any further

increase in pressure, the turning moment of the L.H.S. decreases while that of the R.H.S. increases. The relay cannot therefore tip back again until some mercury has run from the R.H.S. to the L.H.S., i.e. until the pressure in R has fallen *below* breaking pressure.

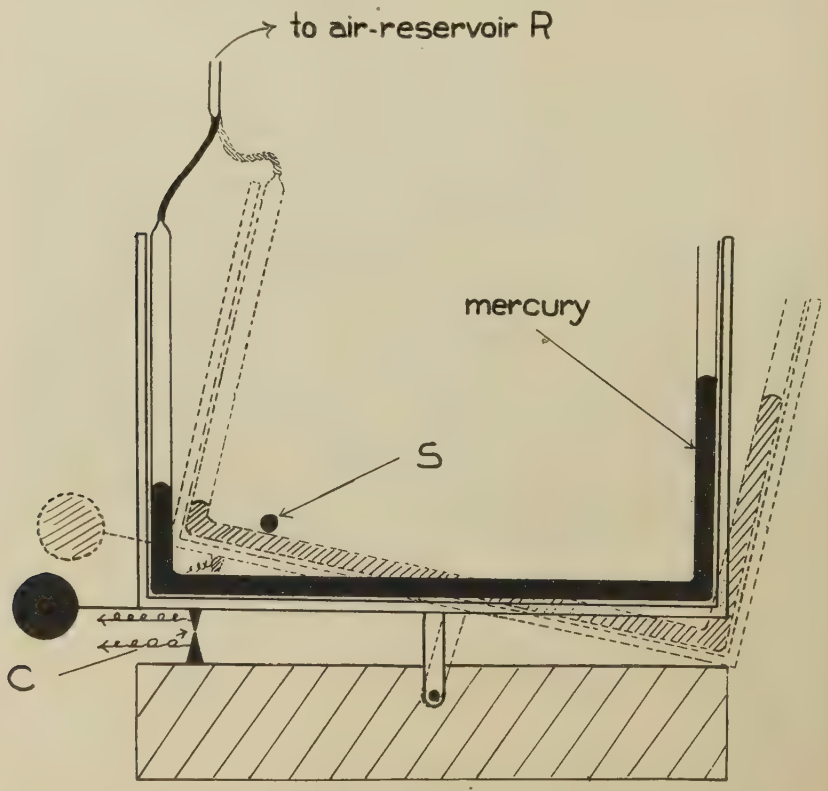

Fig. 3. The tipping barostat relay. Bold drawn diagram shows level position. Feint drawn diagram shows tipped position.

The virtue of this type of relay lies in its 'trigger' action. Once tipping starts in either direction, the forces causing the tipping are automatically augmented by a transfer of mercury to the descending arm and by a C. of G. shift that favours its descent. Therefore both make and break are always quick and the relay never hesitates around breaking point as a result of small fluctuations of pressure in the reservoir R.

Although the performance of such a relay may be precalculated from the size, weight, and allowed tipping angle, of the manometer assembly, the

mathematical relation is so complex that any required performance is best got from such a relay by trial and error. A suitable model for the percolators, built mainly of standard Meccano parts, is shown in Plate 2. The switch 'makes' when a carbon rod, carried by the manometer assembly, dips into a reservoir of salty water in which is immersed another carbon rod that acts as the other pole; the switch breaks when the assembly tips and retracts the rod from the reservoir. This switch satisfactorily controls a 250 watt motor. Its advantages over the more conventional metal-to-metal switches are

1) It cannot corrode
2) The points cannot fuse together
3) It is frictionless.

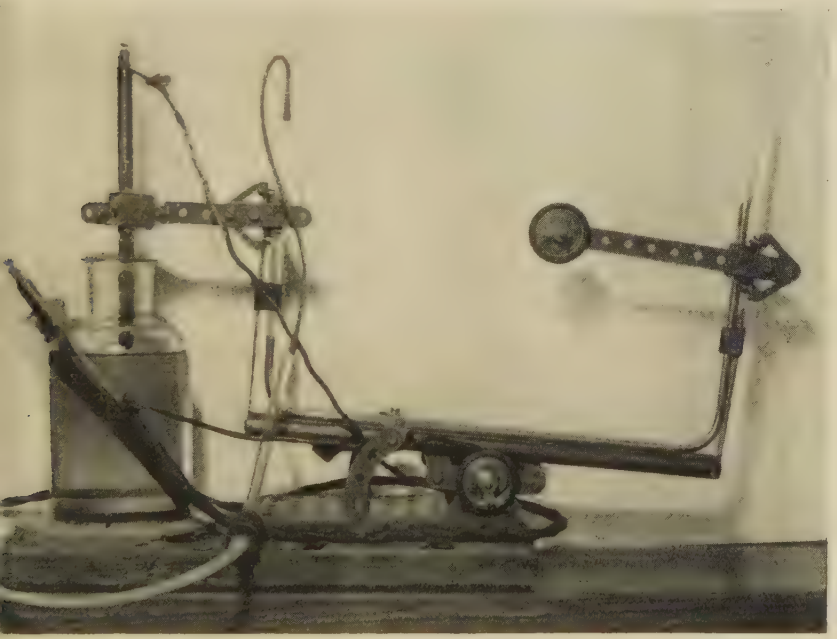

Plate 2. The tipping barostat relay. The wheel and bracket on the R.H.S. are counterweights.

2d. The air-scrubber. Air driving the percolators should be:

a) Almost saturated with water vapour in order to minimize evaporation losses from the reservoir fluid (See Experimental Technique [2]). Complete saturation, which may cause the deposition of water in the supply tubes, is undesirable,

b) Sterilized as far as possible,

c) Freed from traces of ammonia often present in laboratory air.

All three conditions are fulfilled if the air is scrubbed with 5% $CuSO_4.5H_2O$ in 2.5% vol/vol H_2SO_4; a suitable scrubber which offers little resistance to the flow of air passing through it (a desirable feature of a scrubber used in the main air-supply) is shown in Fig 4.

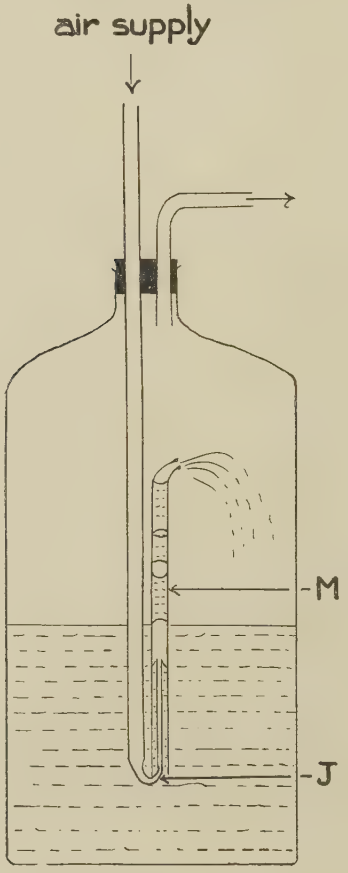

Fig. 4. The air scrubber.

A Winchester quart bottle contains 1 litre of scrubbing liquid. Air to be scrubbed passes from the jet tube *J* (2 mm internal diameter) up the mixing tube *M* (5 mm internal diameter), carrying with it bubbles of scrubbing liquid drawn from the lower part of *M* and projecting them back onto the top of the main bulk of liquid. The air stream is therefore always scrubbed with the least exhausted part of the scrubbing liquid, drawn from the bottom of the bulk. Where it is necessary to remove CO_2 from the air stream (see 'Experimental Technique', Section 4), 1 litre of 50% NaOH in such an

apparatus followed by a soda-lime tower (which not only acts as a spray trap but guards against inadequate scrubbing) should remove all the CO_2 from the air needed to feed 12 percolators for 4 months continuous running.

2e. Screening the percolators. At Rothamsted Experimental Station, England, it was always necessary to screen the percolators very thoroughly against the access of light in order to prevent a visible copious growth of green algae in them. So far this precaution has not been necessary in Trinidad, but the reason for the difference is unknown.

2f. Thermostatic control. The temperature of the soil and percolate is that of the surrounding air. As temperature variations may not only quantitatively affect the speeds of different soil processes but may qualitatively alter the balance between them, some thermostatic control of the temperature of the air round the percolators should be provided in the temperate zones where variations of air temperature are greater than in the tropics. The control need not be fine, 65°F $\pm$ 3°F proved satisfactory in England.

Experimental technique. 1. Soil preparation. Unless the biological effects of drying are to be investigated, soil should always be taken fresh from the site or preserved in a moist condition in the laboratory.

Before use the soil is spread on sheets of paper to dry and then a definite fraction is sieved from it. This fraction must be a compromise between that which gives the maximum weight of sieved material from the original soil and that which percolates most readily. Without exception the most suitable fraction of both temperate and tropical soils has been found to be "passed by 4.0 mm retained by 1.0 mm".

2. Starting and running the percolators.

The percolators are most satisfactorily operated with 10 g soil and 100 ml (initial) percolating fluid. The soil is placed in the soil tube (*S*, Fig. 1) with the *minimum* amount of glass wool at top and bottom, the percolating fluid is measured into the reservoir bottle and the whole assembly (weighing between 500 and 570 g) is weighed to an accuracy of 1 or 2 g. The lift-tube (*L*, Fig. 1) is adjusted to about 0.5 cm above the level of the percolating fluid and the bubbler tube (*B*, Fig. 1) connected to the air-line. As the soil takes up the fluid, the level of *L* is gradually lowered until the soil is percolating satisfactorily. If the soil is a poor heavy clay, the through-put of the percolators should be kept low in the initial stages in order to reduce the

risk of breaking down the soil to a mud. Before a sample of the percolating fluid is taken, the percolator is removed from the air line, reweighed, and distilled water added to the reservoir in sufficient quantity to bring the weight of the percolator to the original weight *minus that of the samples already taken*. This procedure merely compensates for evaporation loss; it is usually inadvisable to keep the percolating volume at the original 100 ml despite the withdrawal of samples because dilution of the percolating fluid alters the distribution of cations between solution and the base-exchange-complex of the soil and may thus affect the biochemical behaviour of the soil.

After the addition of the distilled water the percolator is returned for about an hour to the air line in order to distribute the added water throughout the soil-percolate system. The reservoir fluid is then sampled.

3. Sampling the reservoir fluid. As air movement is always out of the percolator, a pipette sample may be taken from the reservoir, if the main bung is cautiously lifted, with little risk of contamination of the interior of the percolator by air-borne organisms. Although on the analyses of these pipette samples are based all conclusions about such metabolic activities of soil as are reflected as changes in composition of the soil solution (i.e. the solution in the soil tube actually in contact with the soil), it should be realized that in theory the composition of the reservoir solution is not necessarily the same as that of the soil solution. If the concentration of a metabolite being formed or destroyed by the soil is C_s in the soil solution and C_r in the reservoir solution, then percolation with its inherent mixing of the two solutions can reduce the difference $(C_r - C_s)/C_r$ only exponentially. In theory the difference never reaches zero; in fact it approaches zero rapidly.

This was shown by an experiment based on the fact that soil adsorbs methylene blue (MeB) so strongly that when an MeB solution is percolated through soil, the C_s of the MeB can be considered zero.

100 ml of an 0.0025% MeB solution (arbitrary colour value 100) was percolated through 10 g soil under standard conditions. From time to time the MeB concentration of the reservoir solution was determined colorimetrically. A plot of these colorimetric values, and of their logarithms, against the duration of the percolation is shown in Figure 5.

It will be seen that the concentration difference $(C_r - C_s)/C_r$, which in this case is equal to $C_r/100$, is reduced to 1% of its original value in $2\frac{1}{2}$ hours. For all practical purposes, therefore, reservoir analyses do reflect changes in the composition of the soil solution.

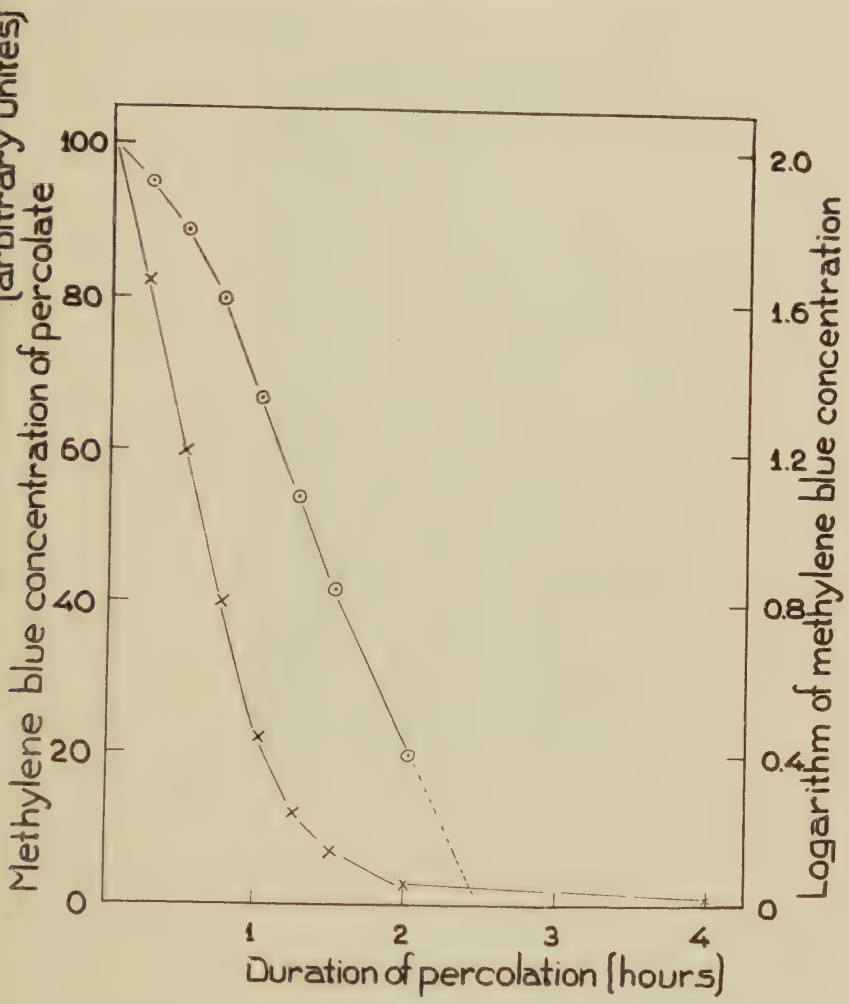

Fig. 5. The removal of methylene blue from 100 ml 0.0025% solution percolated through 10 g soil.
Crosses indicate actual concentrations.
Dotted circles indicate logarithms of concentrations.

4. The measurement of carbon dioxide production by soils. If a soil percolator is driven by an air-supply freed from CO_2 and its exit tube (*E*, Fig. 1) is connected to the bubbler tube (*B*, Fig. 1) of a second percolator containing bartya in its reservoir and glass chips in its soil tube, then the CO_2 produc-

tion of the first soil-percolate system may be followed by successive titrations of the baryta contained in the second percolator. The actual assembly is shown in Figure 6 and Plate 3.

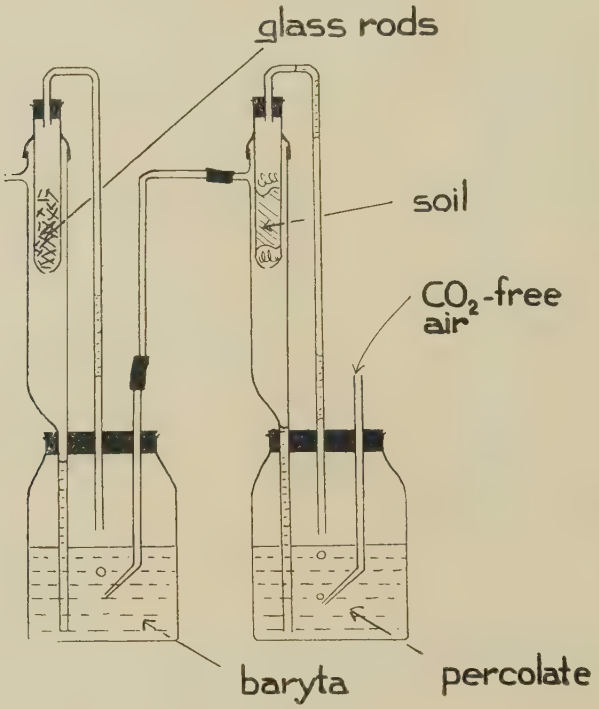

Fig. 6. Assembly for the measurements of carbon dioxide production of soils.

The validity of this, a very recent advance in the application of the percolarion technique, was tested as follows.

In the reservoir of the 'first' percolator was placed 100 ml of approx. M/25 Na_2CO_3 solution, a 50 ml sample of which had been titrated by standard N/10 HCl; in the soil tube was placed approx. 5 g solid benzoic acid. In the reservoir of the 'second' percolator, connected in series with the first, was placed 100 ml of approx M/15 $Ba(OH)_2$ solution, a 50 ml sample of which had been titrated by the same standard acid; in the soil tube were placed 25 1 cm lengths of 2 mm diameter glass rod. When the first percolator was connected to a CO_2-free air-supply, the Na_2CO_3 solution was washed over the benzoic acid and the liberated CO_2 caught in the second percolator. Had the absorption of the CO_2 by the baryta been 100%, then the maxi-

Plate 3. Twin percolators used in carbon dioxide measurements.

mum observed drop in the N/10 HCl titration of 50 ml of the baryta would have been equal to the N/10 HCl titration of 50 ml of the Na_2CO_3. The relevant figures from a number of such experiments are given in Table I. It was found that the maximum drop in baryta titration was reached in about 5 hours.

TABLE I

The collection-efficiency of a percolator

(50 ml samples of Na_2CO_3 titrated by N/10 HCl to methyl orange. 50 ml samples of $Ba(OH)_2$ titrated by N/10 HCl to phenolphthalein)

Na_2CO_3 titration	$Ba(OH)_2$ titrations		Drop in $Ba(OH)_2$ titration	Collection efficiency (%)
	Initial	Final		
18.5 ml	31.6 ml	13.6 ml	18.0 ml	97
18.5 ,,	31.6 ,,	13.2 ,,	18.4 ,,	99
18.5 ,,	31.6 ,,	13.0 ,,	18.6 ,,	100
18.5 ,,	31.6 ,,	13.8 ,,	17.8 ,,	96
22.0 ,,	34.0 ,,	13.4 ,,	20.6 ,,	94
22.0 ,,	34.0 ,,	11.4 ,,	22.6 ,,	103

As the amounts of CO_2 liberated in the above experiments in a few hours represent some days CO_2 production by 10 g of soil, the collection efficiency of the second percolator may be considered adequate.

In actual soil experiments, the baryta concentration is adjusted to the expected CO_2 output and 1 ml samples of the baryta titrated from time to time by approx. N/100 HCl delivered from a 10 ml burette.

5. Soil stimulation. The microbiological population necessary for the performance of a given biochemical process is not usually at its maximum in a fresh soil. When a metabolite is percolated through fresh soil the course of its transformation into other compounds often follows a "bacterial growth curve", i.e. there is an initial lag-phase followed by a sigmoid phase as a microbiological population capable of transforming the metabolite builds up [3]).

If a soil is first percolated with a metabolite until the appropriate microbiological population has been built up, and then washed free of residual metabolite and transformation products by successive percolations with distilled water, the percolation of fresh metabolite (for the experiment proper) may well result in a linear transformation of the metabolite without there being any lag-phase; the soil is then said to be 'stimulated.'

Stimulated soils are useful not only when the effects of poisons or stimulants of a process are to be compared quantitatively [4]), but they may enable one to distinguish between true poisons and bacteriostatics [5]).

6. Accuracy of results. Results obtained from two nominally identical soil-percolate systems may differ from one another because of:

a) inaccuracies of weighing the soil samples or poor sampling technique.

b) inaccuracies in adjusting the soil-percolate volume prior to sampling (see Experimental Technique, Section 2).

c) inaccuracies in pipette sampling of the percolate.

d) inaccuracies in analytical methods.

e) biological variation between soil samples.

Factors *a*–*d* should not contribute more than 2 or 3% to the total variation, which thus resolves itself principally into biological variation between soil samples.

Recent calculations have shown that if one of two nominally identical soil-percolate systems (the soils having been taken from the same bulk sam-

ple at the same time) nitrifying ammonium salts has at time 't' oxidized 'x_1' mg N to nitrate and nitrite, while the other has oxidized 'x_2' mg N in the same way, then either x_1 or x_2 may be expected to differ from the mean of x_1 and x_2 by:

13% ($P = 0.05$, $N = 52$) Temperate soils
20% ($P = 0.05$, $N = 26$) Tropical soils.

Applications and results. Soil processes that can be followed by means of the soil-percolation technique are:

1. Anion formation and destruction. Because, in general, anions are not retained by any exchange complex of the soil, all the anions present in a soil-percolate system are to be found in the percolate; their formation and destruction may therefore be followed directly by percolate analyses (3, 4, 5, 6, 7, 8, 9).

2. Cation formation and destruction. Because all cations are retained to some extent by the base-exchange-complex of the soil, only some fraction of the total cations present in a soil-percolate system is to be found in the percolate; cation formation and destruction can therefore be followed only indirectly by percolate analyses. It is possible to follow the destruction of the ammonium ion during its nitrification because the fraction:

$$r = \frac{\text{Ammonium ion retained by the base-exchange-complex of the soil}}{\text{Total ammonium ion present in the soil-percolate system}}$$

does remain constant throughout nitrification and is independent of the amount of ammonium ion un-nitrified at any time [6]). This technique may be adapted to follow the conversion of an amine to ammonium ions during nitrification of the amine. The experiment is based on the *fact* that both ammonium salts and trimethylamine HCl estimate directly as ammonia-N (i.e. as volatile N) when distilled from NaOH into boric acid in the Markham apparatus [3]), and on the *assumption* that the 'r' for trimethylamine remains constant as does the 'r' for the ammonium ion.

40 g lots of Allotment soil [4]) were percolated in the original percolator [12]) with 200 ml of either $(NH_4)_2SO_4$ or $(CH_3)_3N.HCl$ at a concentration of 140 μg N/ml percolate. In Table II are given the volatile N and nitrate N concentrations of the percolates throughout nitrification of both substances. The 'total (mineral) N' in the soil-percolate system (for simplicity given as the value it would have attained had it all been in the percolate) is calcul-

ated on the assumption that the initially determined 'r' for each system remains constant throughout nitrification.

TABLE II

Nitrification of trimethylamine hydrochlorine and of ammonium sulphate

Compound percolated	'r' (initial)	μg N/ml percolate after (days)						
			1	3	6	8	13	20
ammonium sulphate	0.49	volatile N	72	68	59	44	0	0
		nitrate N	0	15	23	44	121	125
		total N	140	147	138	130	121	125
trimethylamine HCl	0.29	volatile N	100	65	62	49	6	0
		nitrate N	0	10	12	20	101	127
		total N	140	101	99	89	109	127

While the 'total N' as calculated for the ammonium sulphate system falls more-or-less steadily throughout nitrification as a result of denitrification losses, the 'total N' calculated for the trimethylamine system shows a large initial drop followed by a rise to the value finally reached by the ammonium sulphate system.

The simplest explanation of this result is that trimethylamine is converted to some more strongly adsorbed substance, i.e. one of higher 'r', prior to being nitrified. The most obvious substance is the ammonium ion. This inferential result confirms our directly obtained result [7]) that amines are converted to ammonium ions prior to nitrification and cannot be nitrified directly.

3. Carbon dioxide production by soils. Relatively few results of this type are yet to hand (see 'Experimental Technique, Section 4).

Typical results given by 10 g lots of two different soils percolated with 100 ml of either water or 0.1% glucose are shown in Figure 7. Carbon dioxide productions are expressed as mg C evolved/g soil. The soils are:

a) San Juan Soil. A very fertile loam from the Montserrat Hills of Trinidad. S.O.M. 14.0% [10])

b) Rio Claro Soil. A poor heavy clay from the Ecclesville district of Trinidad. S.O.M. 13.0%.

It will be seen that, under the highly aerated conditions obtaining in the percolators, the poor soil tends to lose carbon more rapidly than the fertile soil does.

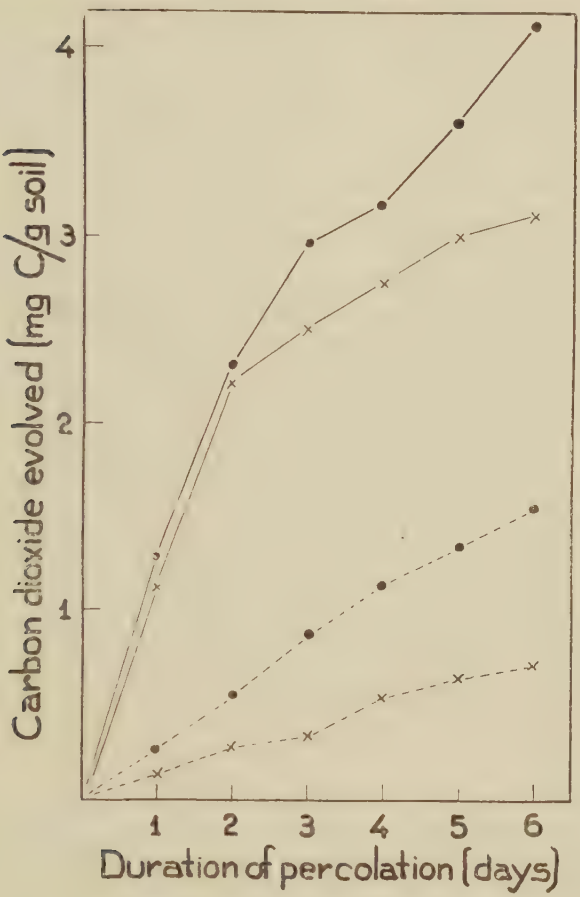

Fig. 7. Carbon dioxide production by 10 g lots of soil percolated with 100 ml of either water or 0.1% glucose.
Crosses indicate fertile San Juan soil.
Dots indicate poor Rio Claro soil.
Dotted curves indicate percolation with water.
Bold curves indicate percolation with 0.1% glucose.

Discussion. This paper is an attempt to present the working of soil percolation technique as a whole. The methods recommended are not the only ones that can be used, nor are they necessarily the best that can be devised; they are however simple and reliable and demand the minimum of complex equipment.

Some of the advantages and disadvantages of the percolation

technique were realized at its inception [3]). To the advantages may now be added a convenient method for measuring the CO_2 output of soils under standardized conditions. The accuracy of measurement is probably no better than 5%, but this should be sufficient for most soil studies such as the rate of evolution of CO_2 from various organic materials percolated through the soil or the effect of added mineral N on the rate. In such experiments N-immobilization may be followed in the 'first' percolator [9]) concomitantly with the measurement of CO_2 evolution in the 'second'.

A disadvantage, not previously mentioned, inherent in the percolation technique is its comparitive crudity, especially when it is applied to studies involving cations. For instance a percolate concentration of 1.0 μg $N\text{-}NH_4$/ml corresponds to a total of 20 μg $N\text{-}NH_4$ present in the system per g soil (Soil weight 10 g, percolate volume 100 ml, 'r' = 0.5). This is equivalent to a dressing of approximately 2 cwt ammonium sulphate per 1000-ton acre. Percolate concentrations may, in practice, be up to 100 μg $N\text{-}NH_4$/ml or even higher.

The soil percolation technique is not new, it is merely an extension of the technique of Schloesing and Muntz [11]). The virtues of the modern technique herein described lie almost entirely in the simplicity, adaptability, and automatic nature, of the apparates used.

Summary

1) Technical details of the operation of the soil percolation technique are fully described.

2) An adaptation of the technique to the measurement of the carbon dioxide output of soils is presented.

3) The technique is discussed.

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INVESTIGATIONS CONCERNING THE PREPARATION AND APPLICATION OF SUBSTITUTES FOR THE PRESENT PRACTICE OF USING STABLE MANURE AND CEREAL STRAW IN THE BIOLOGICAL HEATING OF HOT-BEDS

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I. *Introduction.* The increasing mechanisation of transport and agriculture will entail a shortage of stable manure for horticultural purposes. Already in some regions of Holland it is difficult to procure stable manure for use in the biological heating of hot-beds.

Stable manure with wheat or rye straw is chiefly used in the growing of cucumbers (*Cucumis sativus* L.) and melons (*Cucumis Melo* L.) in forcing frames. Since this method is practised on a large scale in the Netherlands it is worth while looking for a suitable substitute for stable manure for this purpose.

The question may be raised as to how far it would be practicable to use hot water pipes or electrical heating for the frames. Compared with biological heating it would appear that such methods have important disadvantages. Firstly, equipment for use with hot water or electrical methods is still scarce and expensive. Secondly, the importance of the carbon dioxide produced during decomposition of the straw must not be overlooked since an abundant supply of carbon dioxide for use in photosynthesis seems to be of first importance in growing cucumbers or melons; probably because these plants have a large leaf area and a rapid rate of growth. Under normal conditions of temperature and light intensity carbon dioxide concentration in the atmosphere has been shown to be a limiting factor in the rate of photosynthesis in (native) plants; this will hold to an even greater degree for rapidly growing plants such as cucumbers and

melons under forcing conditions. Finally, the use of straw in forcing frames results in the production of a fertile compost which is very suitable for raising seedlings. Biological heating, therefore, offers advantages over methods using hot water or electricity.

II. *Brief survey of the literature.* It appears from the literature that much work has been done on the preparation of fertilisers by composting straw and on the use of straw for the biological production of heat for horticultural purposes. In Germany studies were especially devoted to the preparation of substitutes for stable manure for use in hot-beds, whilst American, French and Russian investigators were more interested in the production of fertilisers, which could be used as substitutes for stable manure, by composting cereal straw. From the German papers it appears that substitutes were sought for both stable manure and cereal straw, both materials evidently being scarce in Germany. These investigations will be referred to later.

During the decomposition of stable manure, or a mixture of straw and added mineral nutrients, two phases can be distinguished. In the first phase of decomposition the temperature of the heap rises rapidly, under certain conditions to 72–73°C, (Norman[2]), Carlyle and Norman[3]) but mostly to 50–70°C (Demolon and Burgevin[4]), Hesse and Schmalfuss[5]), Schulte[6])). Apparently, the first phase of decomposition concerns the utilization of the easily available, water soluble, carbonaceous constituents of the straw; this would explain the great intensity of the process and the high temperatures resulting therefrom. The second phase is characterised by a more gradual release of material from the straw and consequently less intense production of heat. During this phase the straw is converted almost completely to a compost characterised by its high humus content. The pentosan content (Smith, Stevenson and Brown[7]), Flieg[8])), or the C/N ratio (Kappen and Reinhardt[9])), can be used as a measure of the degree of humification of the decomposing straw. When the composting process is complete the pentosan content of the straw is reduced to zero.

From the investigations of Hutchinson and Richards[1]) it is evident that good humification is also obtained when mineral nutrients are added to the straw instead of stable manure. Since 1921

several studies have been devoted to the preparation and properties of such substitutes for stable manure. For further information the papers of Waksman, Fenney and Diehm [10]), Brown and Smith [11]), Waksman and Gerretsen [12]), Demolon and Burgevin [4]) and Ashworth [14]) may be consulted.

For the purposes of the present investigation the first phase of decomposition of the straw, which is characterised by rapid heat production, is the more important. It appears from the German papers quoted above that a rapid and considerable evolution of heat in the first phase is always followed by a more or less complete rotting down of the straw to compost in the subsequent phase.

According to the investigations of Vogel [15]), Reinhold and Hausrath [16]), Schuphan [17]) and Mantel [18]) heat production is governed by the following factors:

1. Heat production in the first phase occurs at the expense of the water-soluble constituents of the straw (cf. also Norman [19])). Hence those kinds of straw having a high content of these constituents will give rise to the greatest heat production in the first phase.

2. Production of heat is strongly accelerated by the addition of ca. 0.7% nitrogen in the form of urea, nitrates, ammonium salts etc.

3. There is no agreement concerning the necessity for addition of phosphates and potassium salts but in order to obtain a good final product, from the point of view of its suitability for use as a fertiliser, the addition of phosphate seems desirable since although straw is comparatively rich in potassium it contains little phosphate.

4. Water must be added to raise the water content of the straw to 70–80%.

In Germany investigations were carried out to ascertain whether materials other than cereal straw could be used in the preparation of substitutes for use as hot-bed manure. For example Vogel [15]) compared cereal straw, tree leaves and peat dust. It was found that peat dust produced little heat and that leaves were inferior to straw in this respect. Reinhold and Hausrath [16]) and Schuphan [17]) also reached similar conclusions. An obvious explanation for the failure in the case of peat lies in the fact that the easily available carbon compounds of the original plant material have already been decomposed during formation of the peat resulting in a high content of lignin-humus compounds. In agreement with this the

peat dust was found to be practically unchanged at the end of the hot bed experiments. It can therefore be said that the German investigators did not succeed in finding a substitute for cereal straw for use in hot-beds.

III. *Laboratory experiments.* In order to get an idea of the heat produced during decomposition of straw, experiments were carried out in vacuum flasks in the following manner Four vacuum flasks were removed from their metal containers and each placed in a wide cylindrical jar, the space of about 2 cm between the jar and flask being filled with glass wool in each case. The effectiveness of the insulation of the various flasks was apparently very variable and for this reason the experiments of each series were carried out successively in the same flask instead of simultaneously in different flasks.

Caraway (*Carum Carvi* L.) and horse-bean (*Vicia Faba* L.) straws were used as well as cereal straw since these crops are cultivated in the northern part of the Netherlands and the straw is not yet used as an industrial raw material.

The effect of the following factors which might be expected to influence heat production during the decomposition of straw was investigated.

1. The most suitable kind of straw from a comparison of microbial thermogenesis with oat, rye, wheat, barley, caraway and horse-bean straws.

2. The influence of the addition of plant nutrients (nitrogen, phosphorus and potassium).

3. Determination of the most suitable source of nitrogen by a comparison of the effects of $(NH_4)_2SO_4$(+ $CaCO_3$), CaNCN, urea and NH_4NO_3.

4. Determination of the minimal quantity of nitrogen necessary for the production of a maximum amount of heat. Comparison of 0.5, 0.7 and 1.0% nitrogen as $(NH_4)_2SO_4$.

5. Determination of the necessity for the addition of inoculation material. Comparison of uninoculated straw and straw inoculated with 5% garden soil or 10% composted straw.

6. Comparison of thermogenesis from cereal straw in the presence of stable manure and mineral nutrients respectively and from straw without added mineral nutrients.

The standards used for comparison were: in 1947, the heat pro-

SCHEME I. ARRANGEMENT OF THE EXPERIMENTS IN VACCUM FLASKS.

	a	*b*	*c*	*d*	*e*	*f*
A (fig. 1)	*oat straw* 3.4% $(NH_4)_2SO_4$, 3% $CaCO_3$, 1% superphosphate, 0.9% K_2SO_4, 5% garden soil.	*winter wheat straw* 3.4% $(NH_4)_2SO_4$, 3% $CaCO_3$, 1% superphosphate, 0.9% K_2SO_4, 5% garden soil.	*rye straw* 3.4% $(NH_4)_2SO_4$, 3% $CaCO_3$, 1% superphosphate, 0.9% K_2SO_4, 5% garden soil.	*barley straw* 3.4% $(NH_4)_2SO_4$, 3% $CaCO_3$, 1% superphosphate, 0.9% K_2SO_4, 5% garden soil.	*caraway straw* 3.4% $(NH_4)_2SO_4$, 3% $CaCO_3$, 1% superphosphate, 5% garden soil.	*horse-bean straw* 3.4% $(NH_4)_2SO_4$, 3% $CaCO_3$, 1% superphosphate, 5% garden soil.
B (fig. 2)	oat straw 3.4% $(NH_4)_2SO_4$, 3% $CaCO_3$ 1% superphosphate, 0.9% K_2SO_4 5% garden soil.	oat straw *2% NH_4NO_3,* 1% superphosphate, 0.9% K_2SO_4 5% garden soil.	oat straw *3.4% CaNCN,* 1% superphosphate, 0.9% K_2SO_4, 5% garden soil.	oat straw *1.5% urea,* 1% superphosphate, 0,9% K_2SO_4 5% garden soil.		
C (fig. 3)	oat straw 3.4% $(NH_4)_2SO_4$, 3% $CaCO_3$, 1% superphosphate, 0.9% K_2SO_4, 5% garden soil.	oat straw *4.8% $(NH_4)_2SO_4$,* 4.2% $CaCO_3$, 1% superphosphate, 0.9% K_2SO_4, 5% garden soil.	oat straw *2.4% $(NH_4)_2SO_4$,* 2.1% $CaCO_3$, 1% superphosphate, 0.9% K_2SO_4 5% garden soil.	oat straw 3.4% $(NH_4)_2SO_4$, 3% $CaCO_3$, 1% superphosphate, 0.9% K_2SO_4, *without garden soil.*	oat straw 3.4% $(NH_4)_2SO_4$, 3% $CaCO_3$, 1% superphosphate, 0.9% K_2SO_4, *10% composted straw*	
D (fig. 4)	oat straw 3.4% $(NH_4)_2SO_4$, 3% $CaCO_3$, *1% superphosphate,* 0.9% K_2SO_4, 5% garden soil.	oat straw 3.4% $(NH_4)_2SO_4$, 3% $CaCO_3$, *without superphosphate,* 0.9% K_2SO_4, 5% garden soil.	oat straw 3.4% $(NH_4)_2SO_4$, 3% $CaCO_3$, 1% superphosphate, *without K_2SO_4,* 5% garden soil.			
E (fig. 5)	summer wheat straw 3.4% $(NH_4)_2SO_4$, 3% $CaCO_3$, 1% superphosphate 5% garden soil.	summer wheat straw *without fertilizer,* 5% garden soil.	summer wheat straw *50% horse manure,* 5% garden soil.			
F (fig. 6)	summer wheat straw 3.4% $(NH_4)_2SO_4$, 3% $CaCO_3$, 1% superphosphate, 5% garden soil.	caraway straw 3.4% $(NH_4)_2SO_4$, 3% $CaCO_3$, 1% superphosphate, 5% garden soil.	caraway straw *1.5% urea,* 1% superphosphate, 5% garden soil.	caraway straw *without N,* 1% superphosphate, 5% garden soil.	caraway straw *without N,* *without P,* 5% garden soil.	
G	summer wheat straw 3.4% $(NH_4)_2SO_4$,	horse-bean straw 3.4% $(NH_4)_2SO_4$,	horse-bean straw *without N,*	horse-bean straw *without N,*		

duction by oat straw with addition of $(NH_4)_2SO_4$, $CaCO_3$, superphosphate, K_2SO_4 and garden soil and in 1948, summer wheat straw with the addition of $(NH_4)_2SO_4$, $CaCO_3$, superphosphate and garden soil.

The arrangement of these experiments is given in Scheme I.

50 g samples of the various kinds of air dry straw were each mixed with 130 ml of tap water in which the additional chemicals were dissolved or suspended. Oat and barley straw had always absorbed the water completely after half an hour, this was not always the case with horse-bean straw. Wetting of the straw was carried out in a room where the temperature was about 17°C i.e. the temperature of the incubator in which the vacuum flasks were afterwards placed. This is important as an initial difference in temperature between flask and incubator will disappear only very slowly, because of the insulating properties of the vacuum flasks. Thus in a case where the temperature of the straw in the flask was initially higher the microorganisms in this flask would get ahead of those in a flask with a lower starting temperature and this might lead to erroneous findings. With a view to obtaining good diffusion of air into the decomposing straw care was taken to pack the straw loosely in the flask. Under such conditions artificial aeration did not increase heat production to any measurable extent and it could be dispensed with. The temperature of the decomposing straw in the flasks was measured by means of thermometers with 15 cm stem length between the bulb and the lowest calibration mark. When the flasks had been filled their necks were plugged with glass wool.

The results of these experiments are given in figs. 1 to 7. The curves show two phases of heat evolution in most cases; one after 1 to 2 days and the second after 4 to 7 days. The first maximum is probably due to the utilization of the water soluble constituents of the straw which will be more easily available to the microflora than the insoluble ones. This supposition is supported by the steep slope of the ascending part of the first phase which indicates a rapid production of heat and hence a very active biological oxidation. In this connection it is of interest that Carlyle and Norman[3]) have also observed two maxima in the heat production by de composing straw. In the adiabatic apparatus used by these investigators the first maximum occurred after 48 hours and the second after about 6 days. The greatest production of heat also occurred in the

first maximum whereas in the second, the heat produced was little more than half that in the first.

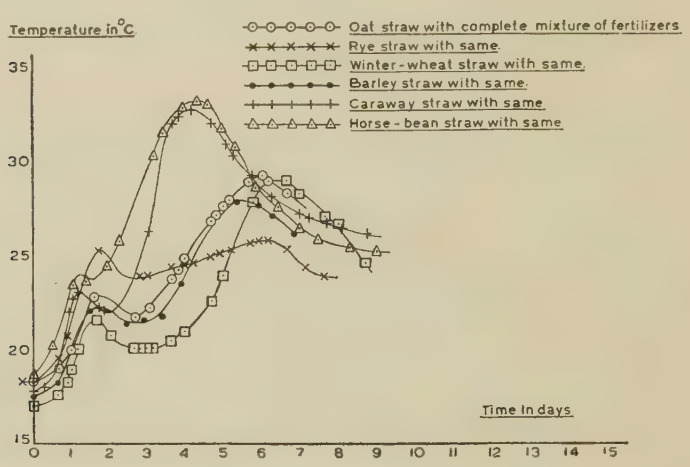

Fig. 1. Heat production in different samples of straw with the same mixture of fertilizers.

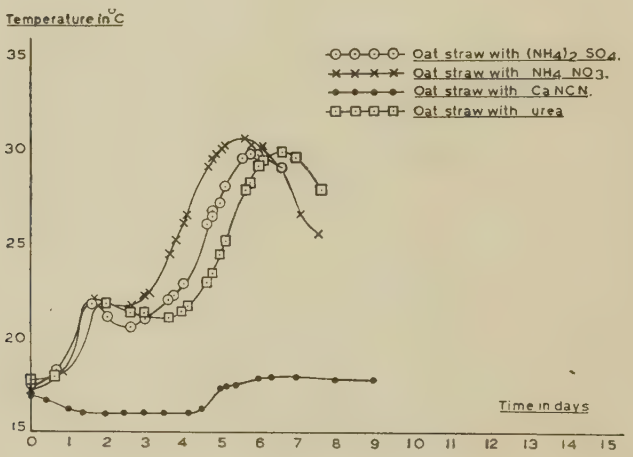

Fig. 2. Heat production in a sample of oat straw with different sources of nitrogen.

The experiments in the absence of additional nitrogen (figs. 5, 6 and 7) show the interesting phenomenon that whereas heat production during the first maximum is not decreased compared with heat

production in the presence of nitrogen, the second maximum fails to appear altogether in the absence of additional nitrogen.

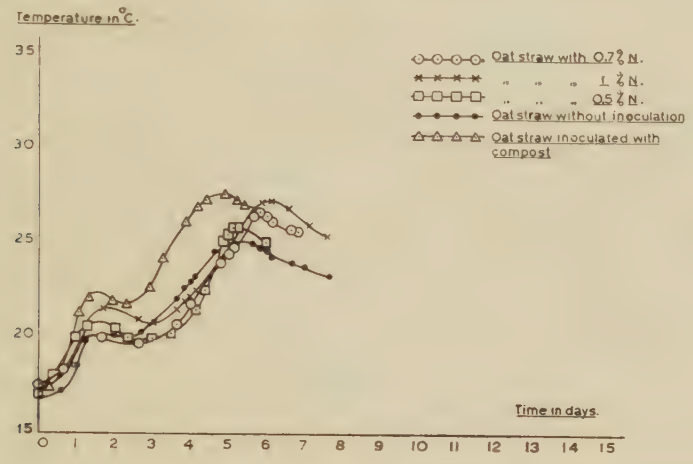
Temperature in °C.
35
30
25
20
15
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
Time in days.
Oat straw with 0.7 % N.
" " " 1 % N.
" " " 0.5 % N.
Oat straw without inoculation
Oat straw inoculated with compost

Fig. 3. Heat production in a sample of oat straw with different amounts of additional nitrogen ($(NH_4)_2SO_4$); influence of adding an inoculating material.

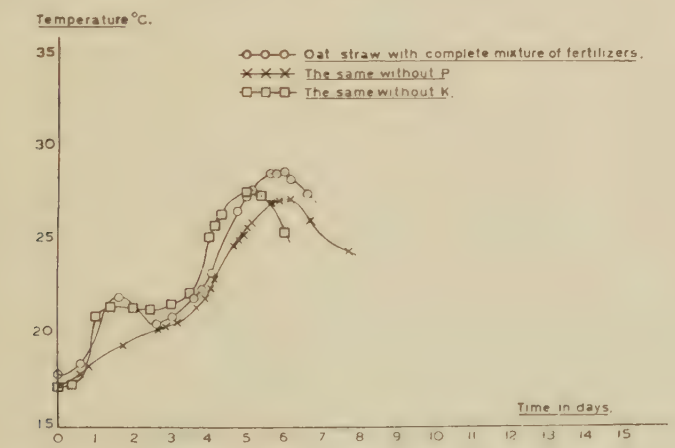

Fig. 4. Influence on heat production in a sample of oat straw of the addition of a source of P (superphosphate) and K (K_2SO_4).

Evidently the decomposition of the less readily available constituents of straw (e.g. the cell-wall carbohydrates) depends on the addition of available nitrogen. As this also holds for horse-bean straw, which is rich in nitrogen (cf. ch. 5), the conclusion may be

drawn that its nitrogen is only partly available to the microorganisms. The following characteristics of heat production from straw may be deduced from figs. 1 to 7.

1. All kinds of straw appear to be suitable as sources of biological heat (cf. fig. 1). In general the samples of cereal straw show a more gradual production of heat than the samples of caraway and horsebean straw although the latter produce more heat than cereal straws.

2. Not all sources of nitrogen give rise to good heat production. As is evident from fig. 2 CaNCN even has a pronounced inhibitory effect on heat production. This is remarkable in view of the rather good results reported by the German investigators (15, 16, 17, 18) using

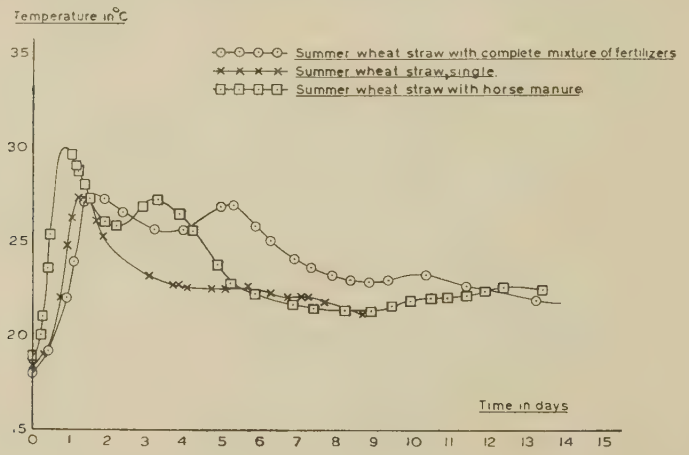
Temperature in °C
Summer wheat straw with complete mixture of fertilizers
Summer wheat straw, single
Summer wheat straw with horse manure
35
30
25
20
15
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
Time in days

Fig. 5. Comparison of heat production in wheat straw with horse manure and additional chemicals resp., and in straw without fertilizers.

this material in field experiments. Our preparation of CaNCN apparently contained a substance which retarded or even prevented the development of microorganisms.

3. It appears from fig. 3 that the differences in heat production in the experiments with varying quantities of additional nitrogen are rather small, but nevertheless perceptible. The largest quantity of additional nitrogen yielded the highest temperatures; also the area enclosed by the curve and the abscissa for $t = 17°C$ (the temperature of the incubator) is somewhat larger when more nitrogen was applied However, the differences in heat production obtained with 0.5, 0.7 and 1.0% of added nitrogen are so small that addition of more than

0.7% N seems uneconomical; this is in accordance with the results of Halverson and Torgerson [20]).

4. It appears further from fig. 3 that inoculation of the straw with garden soil or straw compost has no important influence on the quantity of heat produced (compare the areas enclosed by the curves and the abscissa for $t = 17°C$). In the case of inoculation with compost, heat production starts more rapidly. On the whole, however, inoculation does not seem necessary.

5. Omission of nitrogen from the mixture of mineral nutrients added results in a strong decrease in heat production (figs. 5, 6 and

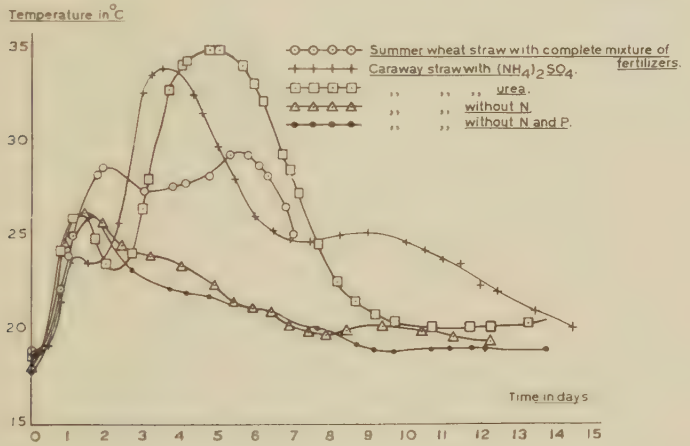
Temperature in °C
35
30
25
20
15
Summer wheat straw with complete mixture of fertilizers.
Caraway straw with $(NH_4)_2SO_4$.
,, ,, ,, urea.
,, ,, without N.
,, ,, without N and P.
Time in days
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15

Fig. 6. Heat production in a sample of caraway straw compared with that in wheat straw; influence of omission of additional N and P.

7). Fig. 4 shows that omission of phosphate or potassium has a much less pronounced effect. Since cereal straw is rich in potassium it is not surprising that its omission produces little effect. The effect of omission of phosphate is small, it is true, but nevertheless perceptible. This is easily understood, as the phosphate content of cereal straw is only small. Consequently it will be advisable to incorporate some phosphate in the preparation of a substitute hot-bed manure, especially as it will result in a higher grade compost being obtained subsequently.

6. A comparison of thermogenesis by cereal straw in the presence of stable manure and mineral nutrients respectively is especially important from the practical point of view. Fig. 5 indicates that a

mixture of cereal straw and stable manure is capable of a short, but very rapid, heat production, whilst a mixture of straw and mineral nutrients gives a more gradual production of heat. Since in practice the stable manure is layered on the straw as a top dressing, and not mixed with it, the opposite is found there (cf. Ch. IV). Cereal straw without mineral nutrients produced considerably less heat. It may be inferred from the curves that to a certain extent heat production is maintained over a longer time. After about 10 days the majority of samples have acquired the odour of forest soil, and it may therefore be expected that after the second maximum of heat production

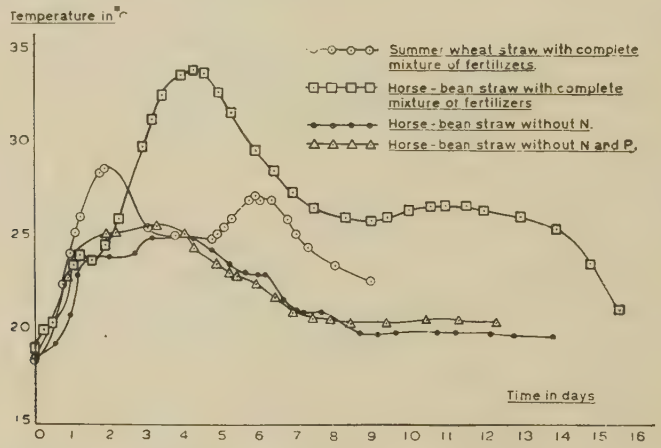
Temperature in °C
35
30
25
20
15
Summer wheat straw with complete mixture of fertilizers.
Horse-bean straw with complete mixture of fertilizers
Horse-bean straw without N.
Horse-bean straw without N and P.
Time in days
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16

Fig. 7. Heat production in a sample of horse-bean straw compared with that in wheat straw; influence of omission of additional N and P.

there is a shift in the composition of the microflora population due to the development of Actinomycetes. These organisms are capable of decomposing cellulose and pentosans completely whereas lignin is only partially converted. Thus a kind of mould or humus remains. Since these latter conversions only proceed slowly, the rate of heat production is small.

From the results of these laboratory experiments the conclusion may be drawn that successful preparation of a substitute for the hot-bed manure in current use seems quite feasible. In principle all kinds of straw seem suitable, but in view of commercial considerations caraway and horse-bean straws seem to be most useful. The addition of nitrogen in the form of urea, $(NH_4)_2SO_4$ or NH_4NO_3 and of super-

phosphate has given considerable heat production. Addition of nitrogen in the form of CaNCN had a detrimental effect. Addition of potassium and inoculation material appeared to be superfluous.

IV. *Experiments under field conditions.* After having established the conditions for optimal heat production by laboratory experiments these results were tested in field trials at the Municipal Horticultural Experiment Farm at Sappemeer (Province of Groningen) *). These experiments were performed in the spring in 1947 and 1948. The 1947 experiments took place under rather unfavourable conditions; the winter of 1946/7 being very severe and persisting late, thereafter passing into a very early and hot summer. Moreover the experiments were hampered by a shortage of soil thermometers. Nevertheless some important conclusions could be drawn from them. In 1948 some of the 1947 experiments were repeated and a number of new ones carried out under more favourable circumstances.

A. The 1947 Experiments. These experiments covered the following points:

1. The most suitable source of nitrogen.
2. Comparison of hot-beds using cereal straw with stable manure, cereal straw with mineral nutrients added and cereal straw alone.

100 frames were available for the experiments, but only 30 could be used for temperature measurements as soil thermometers were not available for the remainder. The 100 frames were arranged in a double row running north to south. Each experiment contained 5 frames, each frame 120 cms in length and 80 cms in width, and was duplicated i.e. 10 frames were used for each point of the experimental programme. Initially the 4 frames at the north and the 6 frames at the south end of the double row were not used in the experiments; subsequently the latter were used for a special purpose. Over the whole length of each of the two rows of frames a trench was dug about 60 cms deep and 80 cms wide. Both trenches were divided into lengths corresponding to 5 frames by means of sheets of eternite. The straw was placed in the compartments of the trenches, soaked with water and trodden down. Subsequently the different mixtures of mineral nutrients were added. To facilitate distribution the mine-

*) The author's thanks are due to Mr. J. W. Wiechers, director of the farm, for his valuable assistance.

ral nutrients for each experiment of 10 frames were mixed with 15 kg of moistened peat dust. In the 1947 experiments the mineral nutrients were washed into the straw until it could be assumed that distribution was more or less homogeneous. In the 1948 experiments the mineral nutrients were washed in only very superficially *i.e.* equivalent to a top dressing. In each case the straw was then covered with a layer of garden soil about 15 cms in thickness. In 1947 the garden soil was added on May 8th and 6 thermometers, with the mercury reservoirs situated at the junction of straw and soil, were placed in the soil the following day. On May 12th one melon plant was planted under each frame. Temperatures were read at 6 o'clock each morning the outdoor temperature being recorded by a thermograph.

The experimental programme was as follows:

1. 150 kg of wheat straw per 10 frames without added mineral nutrients.
2. 100 kg of straw and 120 kg of fresh stable manure.
3. 150 kg of straw with 5.3 kg $(NH_4)_2SO_4$ + 4.5 kg $CaCO_3$.
4. 150 kg of straw with 5.5 kg Ca-Am-Nitrate + 1.5 kg superphosphate + 1.4 kg K_2SO_4.
5. 150 kg of straw with 5.5 kg CaNCN (containing 20% nitrogen) + 1.5 kg superphosphate + 1.4 kg K_2SO_4.
6. 150 kg of straw with 2.4 kg urea + 1.5 kg superphosphate + 1.4 kg K_2SO_4.

The results of the temperature measurements are given in fig. 8 and the following conclusions may be drawn from these data.

In general, substitute for hot-bed manure, as prepared in 1947, produced a more intense heat in the beginning than straw with stable manure. At the conclusion of the experiments, however, straw with stable manure and also straw alone had a somewhat higher temperature. Under the abnormal circumstances of the spring of 1947 the experiment using urea showed a very strong heat production the soil temperature rising to 36° at a depth of 15 cms. After 14 days the heat production appeared to decline and the temperature of the soil fell a little below that in the experiment with stable manure. Similar results were obtained in the experiments with other sources of nitrogen with the exception of CaNCN. This substance appeared to have rather a detrimental action when added to the straw; this is evident from a comparison of the results with the curve obtained for straw

alone, and is in accordance with the results of the laboratory experiments.

On account of the exceptionally hot weather during May 1947 the state of the plants in the different experiments was practically the same.

In the middle of May the outdoor temperature often drops considerably at a time coinciding with the period of minimum heat production; this sometimes results in damage to the crop. Attempts were made to prevent this by addition of a solution of molasses to

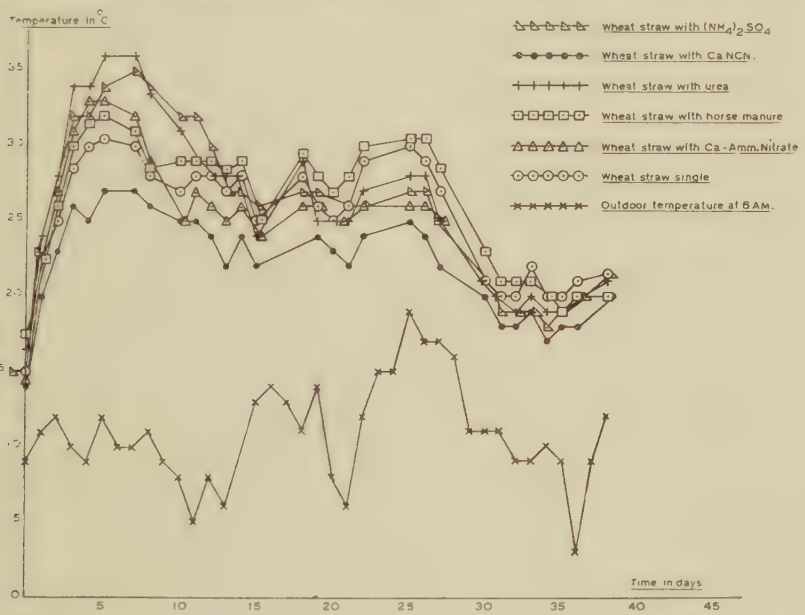

Fig. 8. The temperature changes of the soil in hot-beds heated with different samples of hot-bed manures (Experiment of 1947).

the straw. This was carried out by pouring the solution of molasses into a perforated *T*-shaped tube inserted in the upper part of the layer of straw. The quantity of molasses (dry weight) added amounted to 5% of the original weight of the straw. This initiated a renewed heat production with a maximum temperature difference as compared with the other frames of 7°C which lasted at least ten days. Such a procedure would be sufficient to bridge the cold period in May but how far this would be an economical proposition depends on the ruling price of molasses.

B. The 1948 experiments. Since the spring of 1948 was normal, conditions for the experiments were considerably more favourable than in 1947 and as 20 soil thermometers were now available the investigation could be carried out on a larger scale. The experiments were started on April 7th, when the additional mineral nutrients were distributed over the moistened straw in the trenches, this being about one month earlier than in the preceding year.

With two exceptions the procedure was identical with that of the preceding year.These exceptions were (1) temperature measurements made in an unheated frame *i.e.* without hot-bed material of any kind, and (2) the additional mineral nutrients were washed into the upper layer of straw only, by using only small quantities of water. The latter procedure was adopted in order to obtain a more gradual production of heat than that of the preceding year, when the additional mineral nutrients had been distributed more or less homogeneously throughout the layer of straw. The latter method results in a simultaneous production of heat throughout the mass of straw whereas in the former, the lower layers of straw do not produce much heat until the additional mineral nutrients reach them (cf. the results obtained with a mixture of cereal straw and stable manure in the laboratory experiments mentioned in ch. III sub 6.

Some experiments of the preceding year (1947) were repeated on a more extensive scale, firstly those concerning the most suitable source of additional nitrogen (stable manure, urea, $(NH_4)_2SO_4$, Ca-Am-nitrate) and secondly, those concerning the suitability of various kinds of straw (cereal, caraway, horse-bean and a mixture of these). In these investigations the results obtained were compared with those obtained with straw without the addition of mineral nutrients i.e. straw alone, and with those from a mixture of straw and stable manure.

100 frames in a double row were available, 4 frames at the north end being left out and 6 at the south end of the double row serving as a blank *i.e.* without any heating.

The following experiments were carried out in 1948. Unless otherwise stated the quantity of straw per frame was 15 kg.

1. Unheated frames. The soil temperature of these frames subtracted from those of the other sets of frames gave the effective heat production.

2. Summer wheat straw without added mineral nutrients (i.e. straw alone).

3. Summer what straw (10 kg per frame) with stable manure (20 kg per frame).

4. Mixture of the various kinds of straw (5 kg caraway straw below, 5 kg cereal straw in the middle and 5 kg horse-bean straw on top per frame).

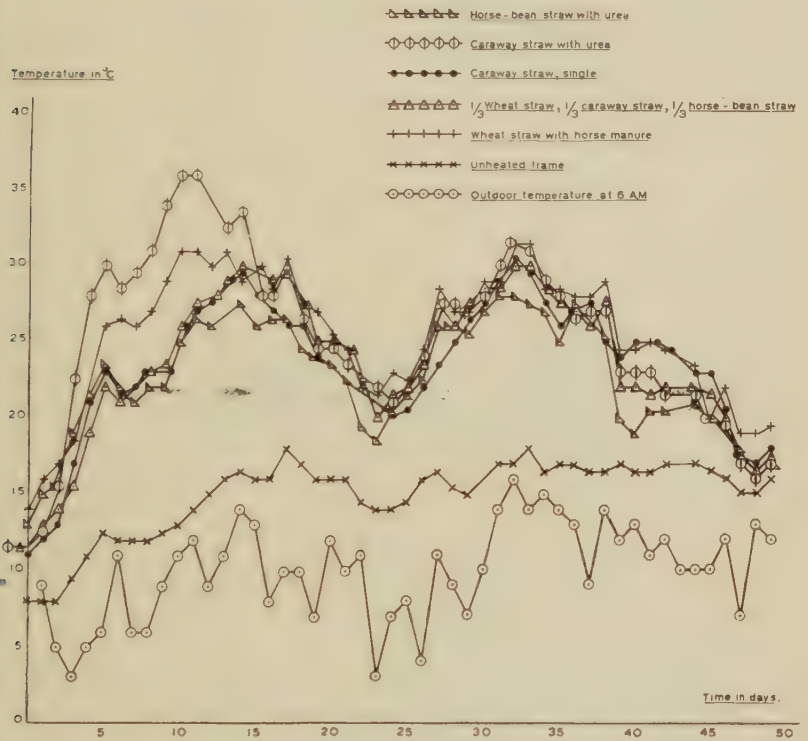

Fig. 9. The temperature changes of the soil in hot-beds heated with different samples of hot-bed manures (Experiments 1948).

5. Caraway straw without additional mineral nutrients (*i.e.* caraway straw alone).

6. Caraway straw with 0.7% added nitrogen in the form of urea (2.4 kg per 150 kg of straw) and 1% superphosphate.

7. Summer wheat straw with same additions as in 6.

8. Summer wheat straw with 0.7% nitrogen in the form of

$(NH_4)_2SO_4$ and $CaCO_3$ (5.3 kg and 4.5 kg respectively per 150 kg of straw) and 1% superphosphate.

9. Summer wheat straw with 0.7% added nitrogen as Ca-Am-nitrate and 1% superphosphate.

10. Horse-bean straw with same additions as in 6.

Each experiment was duplicated, the total number of experiments being 20. In 1948 too melon plants were cultivated in the hot-beds, and after about 14 days a distinct difference in size of the plants was observed, those on some hot-beds having a leaf spread of only 20 cm and those on others up to 40 cm.

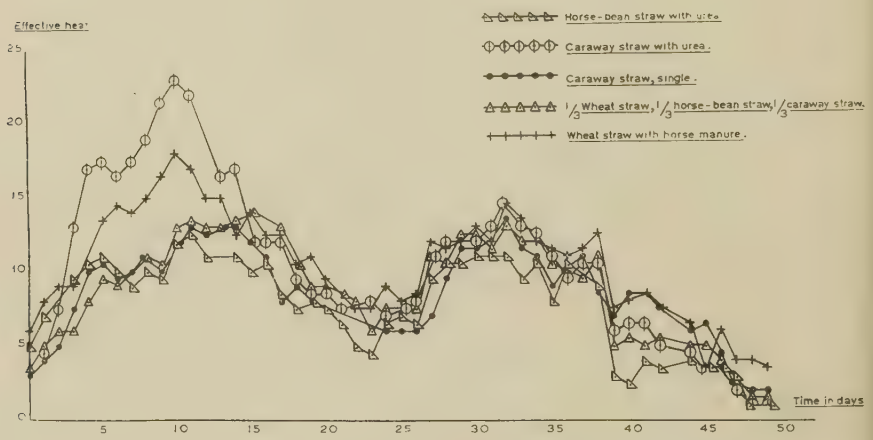

Fig. 10. The temperature changes of fig. 9 corrected for the temperature of the unheated frame.

Soil temperatures at a depth of 15 cm during the course of the investigation are given in fig. 9. Since the temperatures in the unheated frames were available in 1948 it was possible to correct the temperature readings for that due to the glass covering by subtracting the temperature of the unheated frames from those over the various hot-beds (cf. fig. 10).

From figs. 9 and 10 it appears that caraway straw and urea constitutes an important improvement over the traditional mixture of cereal straw and stable manure. During the first 14 days the temperature of the former mixture is even 4°C to 6°C higher. On the other hand, caraway straw alone, a mixture of equal parts of caraway, cereal and horse-bean straw, and horse-bean straw with urea added,

all have a considerably lower temperature than wheat straw with stable manure added. This does not agree with the favourable results obtained with horse-bean straw in the laboratory experiments and the discrepancy is difficult to explain. Fig. 11 shows the result obtained in the duplicate experiments. Apart from the fact that the frames of this series produced, on the whole, somewhat less heat than those of the first series the results are similar to those shown in fig. 10. Heat production in caraway straw with added urea is still about 4°C higher than that in the other experiments of this series, even during the 5th week of the experiment. After the 5th week heat production

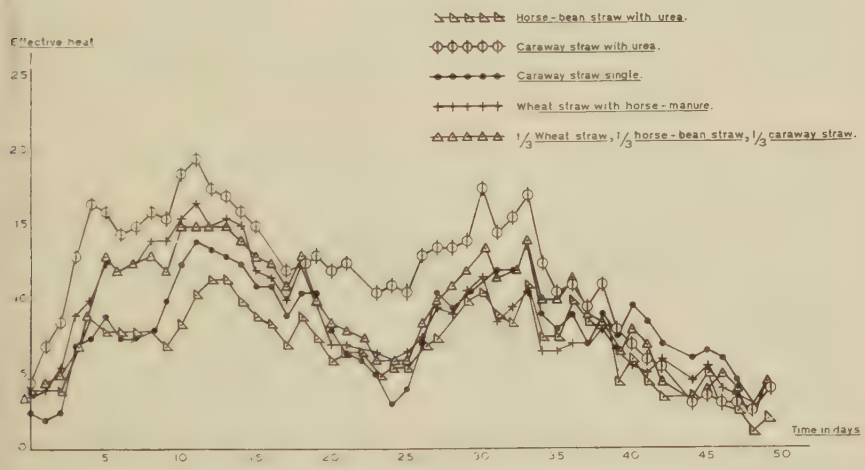

Fig. 11. The results of duplicate experiments of those in fig. 10.

decreases to the same level as that of the other experiments in the series.

Fig. 12 demonstrates that the mixture of wheat straw and urea was also decidedly warmer than the wheat straw with added stable manure. To economise space the duplicate of fig. 12 has been omitted. It also follows from figs. 9, 10, 11 and 12 that, under the conditions of the experiment, heat production ceases after the 7th week. Assuming this to be a characteristic sequence of events and taking into account the cold days which often occur in the middle of May, this would mean that forcing frames should not be started before the end of March. Further, two maxima of heat production occur, the first after 10–15 days and the second after about 30 days. Whilst the

height of the first maximum varies considerably for the different mixtures the second shows but little variation. It is, therefore, improbable that this second maximum corresponds to the second maximum of heat production in the experiments with vacuum flasks. It may be partly due to the 2° to 3°C increase in average day temperature which occurred during this period and which raises the rate of decomposition of the plant material to some extent. However the influence of the change in composition undergone by the microbial population during the course of time will have a much greater effect. It could be shown that in the first period of decomposition moulds and bacteria predominate whereas in the second phase Acti-

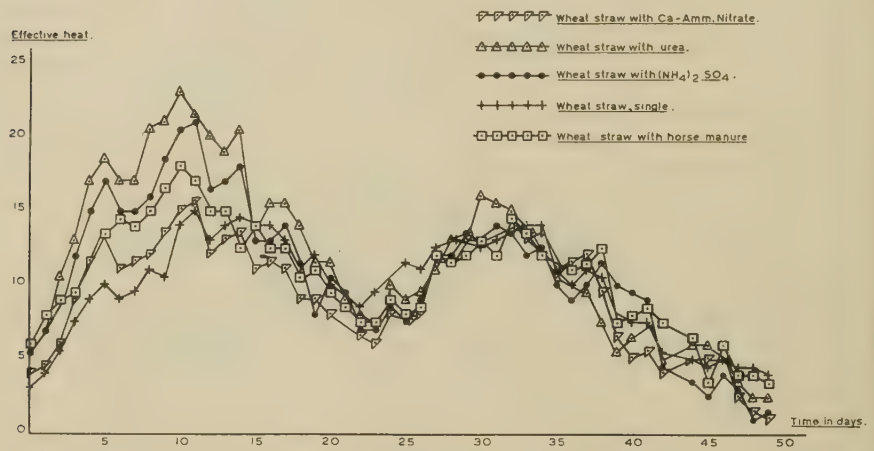

Fig. 12. The results of temperature changes in some samples of cereal straw with different fertilizers.

nomycetes prevail and the latter phase is accompanied by the typical small of fresh earth.

From a comparison of the results of the field experiments of 1948 with those of 1947 it may be stated that in general the results obtained in 1947 appear to be reproducible. This holds good especially for the combinations of wheat straw and urea, which showed maximum heat production in both years. In 1947 the mixtures of straw and mineral nutrients produced more heat initially than the usual combination of wheat straw and stable manure but at a later stage the wheat straw and stable manure combination showed a somewhat higher temperature. Since the heat production in the later stages is

rather important from the point of view of occurrence of cold nights in the middle of May this would mean a disadvantage for the straw and mineral nutrient mixture. However, as shown above in 1948 this drawback was successfully overcome.

It is evident that the combination of caraway straw and added urea (and also some superphosphate in order to obtain a good compost afterwards) is the most suitable substitute for hot-bed manure. As well as producing more heat than the other substitutes investigated it is also cheaper since industry is not yet interested in caraway straw. Cereal straw with added urea is also a promising substitute for the traditional combination of cereal straw and stable manure; its use is also economically possible on account of the low cost of the small quantity of urea required for good heat production.

V. *Concluding remarks.* It was observed in chapter III that caraway and horse-bean straw in vacuum flasks gave rise to a more rapid and larger evolution of heat than cereal straws. This may be explained as follows. It has already been commented on that the prevailing opinion in the literature is that heat production occurs chiefly due to utilization of the water-soluble constituents of the straw. The following figures may help to give an idea of the quantities of substances likely to be found in an aqueous extract of straw. A sample of rye straw was found to contain 10.4% of water extractable material. This water extractable material contained 7% reducing sugars, 10% protein, 12% acetic acid, 2½% pentosan, 11% "lignin" *) and 35% ash. The remaining 22½% may have contained non-reducing carbohydrates, non-volatile organic acids, etc. Among these substances the sugars and acetic acid are undoubtedly attacked most readily by microorganisms and are thus probably, to a large extent, responsible for the first maximum of heat production (cf. figs. 1 to 7). The second maximum may be produced by oxidation of other constituents of the aqueous extract possibly together with some of the lower molecular weight insoluble pentosans (cf. Carlyle and Norman [3]).

The content of water extractable material in the various kinds of straw used were: horse-bean straw 18.0%, caraway straw 14.9% and summer wheat straw 12.5%, and this to some extent accounts for the

*) i.e. material remaining undissolved after treatment of the residue, obtained by evaporation of the extract, with 72% H_2SO_4 for 2 hours at 20°C.

more rapid and greater heat production with caraway and horse-bean straw than with cereal straw. As already mentioned in ch. III all the kinds of straw investigated showed two periods of heat production when additional nitrogen had been added. In the first period heat production was more rapid than in the second. Similar findings were obtained by Carlyle and Norman[3]). These investigators further found that straw treated with hot water still gave two maximà of heat production. Apparently the more easily available constituents were not completely removed by this treatment. They also found that straw treated with dilute NaOH gave only one maximum which corresponded with the second maximum observed in the present investigations. The treatment with NaOH apparently removed all easily available constituents from the straw.

Omission of added nitrogen in both the present experiments, and those of Carlyle and Norman resulted in the non appearance of the second maximum. Apparently the small amount of available nitrogen originally present in the straw is used up completely in the initial heat production which takes place during the utilization of the more available carbon compounds in the straw. At first sight it is somewhat surprising that this also holds good in the case of horse-bean straw which has a much higher nitrogen content than cereal straw. Apparently, of the total nitrogen content, reckoned as 7% protein, only 2.8% was soluble in hot water. The corresponding percentages were 3.5 and 0.8 for caraway straw and 4.5 and 1.9 for summer wheat straw. It would seem that most of the nitrogen present in straw is in an insoluble form. It will be seen from fig. 7 that the quantity of heat produced by horse bean straw without added nitrogen is greater than that produced by either caraway straw or wheat straw under the same conditions. A higher content of water soluble nitrogen in the straw apparently results in a greater heat production.

Carlyle and Norman[3]) point out that in their experiments with vacuum flasks the reproducibility of results was poor. In the present experiments satisfactory reproducibility of results was obtained. The curve of heat production was observed to become more irregular when the temperature rose above 40°C; this was only observed once and in a flask not used for other experiments. This may be due to a change in the microbial population above this temperature, thermophilic organisms replacing the mesophilic orga-

nisms which develop at the beginning of the experiment. Whilst in the experiments of C a r l y l e and N o r m a n the initial temperature in the vacuum flasks was 25°C, in the present experiments the initial temperature in the vacuum flasks was 17°C. Thus the "critical" temperature of 40°C was reached more often in the experiments of the former investigators than in those of the present investigators; this may explain the poor reproducibility of results mentioned by these American investigators; further it is possible that their vacuum flasks had better insulating properties than ours.

Summary

In view of the threatened shortage of stable manure for the preparation of hot-bed manure in Holland, the suitability of various substitutes was investigated both by laboratory and field experiments.

In the laboratory experiments it was found that all the kinds of straw investigated (oat, barley, wheat, rye, caraway and horse-bean) are capable of giving rise to rapid heat production after wetting with a solution of mineral nutrients. The greatest and most rapid heat production was obtained from caraway and horse-bean straw.

With all kinds of straw heat production curves having two maxima, after 1 to 2 and 4 to 7 days respectively, were obtained.

The most suitable sources of additional nitrogen (at the rate of 0.7% N calculated on the basis of the initial dry weight of the straw) proved to be urea, $(NH_4)_2SO_4$, $CaCO_3$ and NH_4NO_3. CaNCN appeared to be unsuitable on account of some toxic action.

A mixture of cereal straw and stable manure gave a very rapid heat production though it was of comparatively short duration.

Omission of nitrogen from the mixture of added mineral nutrients had no effect on heat production during the first maximum but under these conditions the second maximum failed to appear.

The addition of a source of phosphorus (superphosphate) or potassium (K_2SO_4) proved to have hardly any effect on the quantity and rate of heat production.

In view of the fact that cereal straw is poor in phosphorus and rich in potassium addition of the former seems desirable in order to obtain a balanced fertile compost subsequently.

The results of the field experiments were on the whole similar to those obtained in the laboratory experiments. The use of CaNCN as an additional source of nitrogen proved unsuitable. Urea yielded very favourable results both with cereal and caraway straws whilst horse-bean straw gave less favourable results.

Top dressing was found to give better results than mixing the additional mineral nutrients throughout the straw, since in the former case the production of heat is limited to a certain extent by the gradual penetration of

the additional mineral nutrients into the deeper layers of the straw. By this means the vigorous initial heat production is reduced and the period of heat production is increased so as to bridge any cold days which are liable to occur in the middle of May.

The field experiments have therefore shown that the application of a substitute for hot-bed manure, consisting of cereal or caraway straw with urea added, is not only quite feasible on a large scale, but also constitutes a more economical procedure than the present practice of employing cereal straw with a top dressing of stable manure.

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AN OBSERVATION UPON THE INFLUENCE OF THE CLIMATE ON THE EFFECT OF POTASSIUM FERTILIZERS ON GRASSLAND

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Introduction. It is a well-known fact that the effect of potassic fertilizers in cold, rainy summers with little sunshine is greater than in warm, sunny years. The experiments with wheat at Rothamsted have clearly proved this. Fieldcrops need the whole growing season to give a harvest and, hence, it is very difficult to separate the different climatic factors, because in Western Europe wet summers are also cold summers with little sunshine.

With grass we have the opportunity to get more harvests in one year and so it is possible to separate different factors. We made two grassland experiments with different potassium manuring where 5–6 outs in a year were taken. The experiments were made on peat soils near Breukelen in the province of Utrecht. The soil contained in the upper strata (4 inches) 30% organic matter and 45% clay, the pH was 5.5. Both plots were dressed with 160 kg N per year per ha in 5 doses and 1000 kg basic slag and different amounts of potassium were applied in February. The first experimental plot was grazed at intervals of one month on the average with a variation of 3–6 weeks. Before the fields were grazed, 4 small plots were cut and the grass was weighed. The second experiment was conducted over the whole season at intervals of 4 weeks. Further details of these experiments are published in „Verslagen van Landbouwkundige Onderzoekingen" *).

The experimental plots were harvested during the three years 1941–1943 and the results showed that in spring and autumn the yield increased markedly as a result of the potassium supply but in

*) In the press.

summer the effect was negligible, and sometimes even negative.

In figure 1 are given the average yields in these three periods. We see here that the curve of the 5th and 6th cuts differs very much from the curve of the first and second cuts.

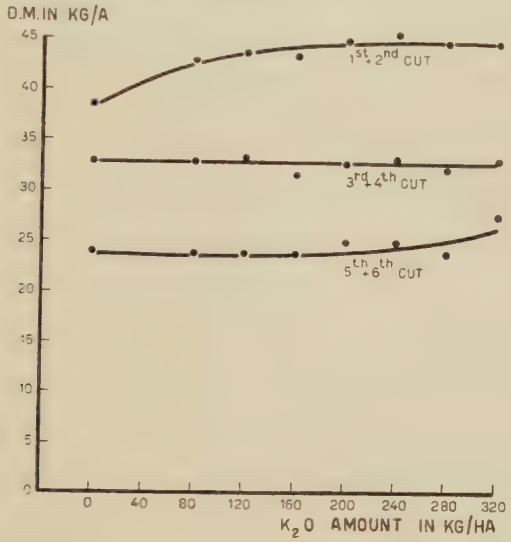

Fig. 1. Yield in kilogram of dry matter per are in different cuts of plots with different potassium fertilization.

In autumn we found only an after-effect of the largest dressings and it is not certain that the optimum had been reached. We have not met this aftereffect before and the reason that it has not been observed before is to be found in the fact that dressings of as much as 320 kgs K_2O per hectare are only rarely applied.

This remarkable influence of the season on the effect of the fertilizer justified further investigation. On the experimental field meteorological observations were not made. To study the influence of the climate we have used the data of the Royal Netherlands Meteorological Institute at de Bilt. The distance between the experimental field and this station is 16 km.

Relation between the effect of the potassium fertilizer on the dry matter yield and the climatic conditions during the growth of the grass. On the grazing experimental plot 18 harvests were cut and on the mowing plot 17. The effect of the potassium fertilizer is indicated

by expressing the yield of the plot without potassium in terms of percentages of the yield of the plots with optimum potassium supply as determined from the three years of the experiments. On the grazing experimental plot this optimum was 60 kg K_2O per ha, for the mowing plot the average of the amounts of 240, 280 and 320 kg K_2O per ha was taken. Only the yield of the plot with 320 kg K_2O of the 6th cut of the third year of the mowing experiment was taken because the lower amounts caused no reaction. We think this is permitted because in autumn the highest comes nearest to the optimum as is shown in fig. 1.

We then determined by means of graphs if there was any relation between the relative yield of the plots without potassium and the climatic factors during the growing period of the grass of the different cuts.

For the first cut in spring we supposed that the growth of the grass began on April 1st.

We found some relation between the average day time temperature, the percentage of sunshine and the average number of sunshine hours per day. The percentage of sunshine indicates the number of hours of sunshine as a percentage of the day length.

The correlations of the yield data with these climatic factors were calculated with the following results:

Grazing experiment $n = 18$

$r_{ot} = \underline{\underline{+ 0.70}}$ $\quad r_{ot.s} = \underline{\underline{+ 0.67}}$ $\quad r_{ot.h} = \underline{\underline{+ 0.63}}$

$r_{os} = + 0.43$ $\quad r_{os.t} = + 0.35$

$r_{oh} = \underline{+ 0.46}$ $\quad r_{oh.t} = + 0.28$

Mowing experiment $n = 17$

$r_{ot} = \underline{\underline{+ 0.63}}$ $\quad r_{ot.s} = \underline{\underline{+ 0.72}}$ $\quad r_{ot.h} = \underline{\underline{+ 0.78}}$

$r_{os} = - 0.23$

$r_{oh} = - 0.11$

n = number of yields
o = relative yield of the plots with potassium
t = daytime temperature
s = percentage of sunshine
h = hours sunshine per day

The correlations with a level of significance of 95% are underlined. When this level is 99% we have given a double underlining. We see from these experiments that the effect of the potassium manuring is positively related to the temperature. This was also found when the percentage of sunshine and the hours of sunshine were taken as constant. It must be stressed that the two experimental plots were laid out in the same years and on the same type of soil. The calculation, however, was done using the temperature of the air at a distance of 16 km from the plots. It is likely that the correlation coefficients with the temperature of the soil of the experiments would have been higher.

The coefficients with the sunshine are less high and are opposite in sign in the two experimental plots.

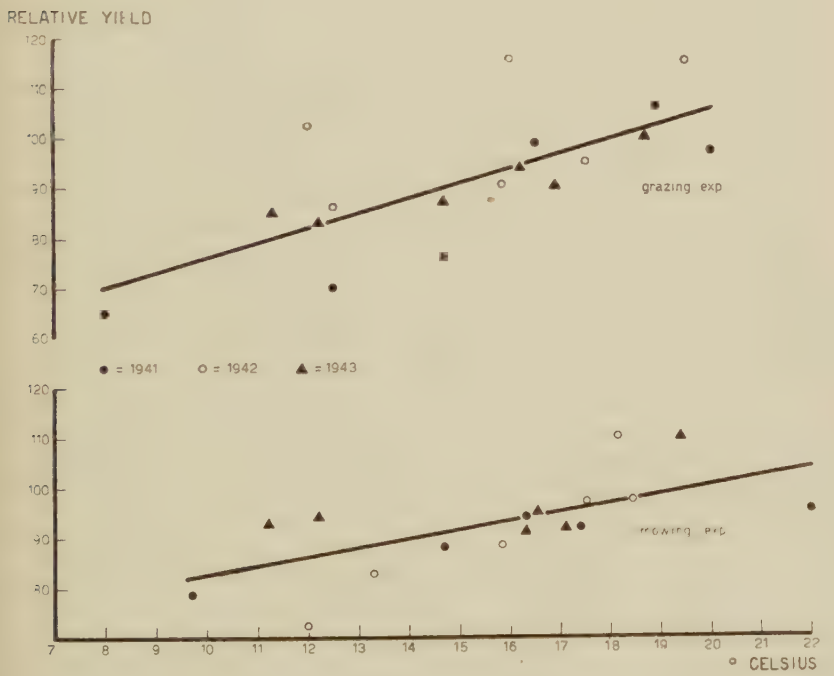

Fig. 2. Relative yields of the different cuts of the plots without potassium fertilization in relation to temperature.

In fig. 2 is given the relation between the relative yield and the temperature. We have calculated the linear regression and have found the following formulae:

Grazing experiment . . . . . $0 = 92 + 2.96\ (t - 15.2)$

Mowing experiment . . . . . $0 = 93 + 1.76\ (t - 15.7)$

The influence of the temperature is important in both experiments so that the different reactions in summer, spring and autumn can easily be explained by this influence.

The relation between the temperature during the growing period and the percentage K_2O in the grass. We have explained already that the different reaction in consecutive cuts during the season was mainly influenced by the temperature during the growth of the grass. It seems likely that there will also be a relation between the temperature and the K_2O percentage in the grass. The higher yield when the temperature is higher would be caused than by the fact that the plants have assimilated more potassium when fertilized. Now it is rather difficult to control this effect on grass because the grass is cut when it not yet ripe and it is known that the percentage K_2O in the dry matter decreases when the grass grows older. To make the different cuts comparable they ought to be cut in the same stage of the growth but this is difficult to do in permanent grassland because the species and varieties differ in earliness of development. The development in the various months is also very different. It is possible to take the percentage of crude protein as a measure for the stage of the growth. In a former publication [3]) we found in a large number of K_2O analyses in grass that the K_2O percentage decreased 0.175% when the percentage of crude protein decreased 1% over a variation of the crude protein from 10-22%. The percentages of crude protein did not vary very much in the two experiments under discussion. We have made the K_2O percentages of all the cuts comparable with grass of 20% crude protein.

In fig. 3 are given the corrected K_2O percentages of the different cuts of the plots treated with potassium fertilizer. Here we see a marked difference between the two experiments. On the grazing experimental plot the K_2O percentage was as high in spring as in autumn, and in summer it was higher. Between the three years there was little difference. In the mowing experiments the percentage increased from the first to the second cut; afterwards it decreased slowly, and in the 5th and 6th cuts the decrease was more pronounced. The percentage decreased also during the three years

of the experiment. We see here the influence of the removing of high amounts K_2O when the grass is only mowed. When the field is

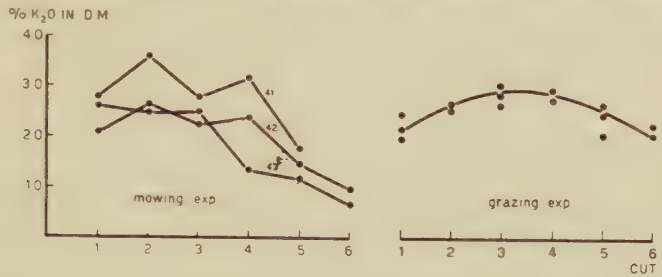

Fig. 3. Percentage of potassium in different cuts on the plots without potassium fertilization.

grazed the K_2O content is rather constant because 90% of the K_2O in the grass comes back in the soil in the urine.

In the grazing experiment we found higher K_2O percentages in summer than we first thought would be the case. We found the following correlation coefficient and regression formula between K_2O percentage (K) and the temperature during the growth of the grass (t). The number of observations was 15.

$$r_{Kt} = \underline{\underline{+ 0.69}} \qquad K = 2.64 + 0.073\,(t - 15.1)$$

The correlation coefficient may be considered reliable and the regression formula shows that the influence of the temperature on the K_2O percentage is not without importance. At 10°C we find 2.10% and at 20°C 2.84%.

In the mowing experiment the withdrawal of the K_2O was a dominant factor so that the percentage decreased during the experiment. We found here the following correlation coefficient:

$$r_{Kt} = + 0.139$$

This coefficient is not significant. It is likely that the correlation would have been much stronger if the decrease by withdrawal was taken as a correction factor. It is, however, not likely that this correction would be linear, so that we have not made use of it. Here the unsatisfactory fact remains that the K_2O content of the plots without K_2O fertilizer decreased rather much during the three years of the experiment, whereas the difference in yield with plots

with high K_2O amounts did not increase during the experiment. The experimental field was grazed several years before the beginning of the trial and the botanical composition changed rather much as a result of the altered treatment. It is possible that the potassium requirements changed with the botanical composition.

Conclusion and summary

The results of the two experiments on permanent grassland on peat soil with different potassium applications were discussed. It was shown that the reaction of the grass to the K_2O dressings depended on the temperature during the growing period. At a temperature of 10°C the plots without K_2O remained 20% behind and at a temperature of 19°C and higher the plots without potassium gave a yield which was as high or higher than with an application of potassium. The correlations showed that in this case the sunshine had little if any influence on the reaction.

On the experimental plot which was grazed the K_2O percentage in the grass increased with increasing temperature during the growth of the grass. On the experimental plot which was mowed this reaction was not marked, due to the strong withdrawal of K_2O in the harvest at each cutting.

Before the second world war, there was a tendency in the Netherlands to give the grassland after a potassium dressing in winter or spring, a light dressing after the first cut for hay. In 1938-'40 several experiments on this subject were laid out but they gave no clear results (Frankena[1]), 't Hart and v. d. Paauw[2])). The second dressing was mostly given in June or July but as a result of the present experiments it will be better to study the effect of a potassium dressing at the end of August on the growth in autumn.

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ASPECTS OF ANTAGONISMS BETWEEN MICRO-ORGANISMS IN SOIL *)

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Introduction. It is now well established that there are many kinds of bacteria, actinomycetes and fungi in soil which are antagonistic to other micro-organisms. Recognition of this has directed intense study in recent years to the isolation of organisms capable of elaborating antibiotic substances active against human and animal pathogens and some of the more important antibiotics now employed for therapeutic use are metabolic products of various species whose normal habitat is that greatest reservoir of micro-organisms, the soil. However, emphasis on the therapeutic application of microbial antibiosis, which ceases to be a problem in soil microbiology following the isolation of the active organism, has tended to obscure the importance of less spectacular, though fundamental microbial antagonisms manifest in soil and their possible relation to soil fertility and the control of plant pathogens.

There is increasing evidence of the existence of microbiological equilibrium in soil, though one ever changing under the influence of temperature, moisture, soil treatment and cropping system. There is, furthermore, evidence that apart from the consideration of the supply of suitable nutrients, the microbiological balance is affected by the antagonistic effects exerted on certain micro-organisms by others. While associations and antagonisms are known to exist between members of the indigenous, saprophytic soil microflora, not concerned with pathological processes in plants, more attention has been given to exploring the antagonism of soil-inhabiting micro-organisms to plant pathogens, stimulated by recognition of the

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practical importance of any successful biological control method based on the inoculation of soil, seed or plants with cultures of antagonistic types. For a survey of this field reference may be made to the most recent discussion by Waksman [9]) and to other reviews on the subject [1]), [2]), [6]), [7]), [8]), [11]).

In the main, attempts at the practical control of pathogenic organisms by application of cultures to seed or soil have not been attended by any marked degree of success. This is believed to be due largely to the inability of antagonists, active against various pathogens in pure culture *in vitro*, to exert their effects in soil where they are confronted with an extensive, mixed microbial population and so exposed to antagonistic effects of other organisms.

Experimental. The manifold antagonisms existing in the mixed microbial population of a soil are suggested by experiments confined to a group of actinomycetes isolated from the rhizosphere of potato in connection with investigations by Rouatt and Atkinson [5]) on the effect of incorporating cover crops on the incidence of potato scab and the microbiological balance in the soil. In view of the favorable effect of soybean crops noted by these investigators, actinomycetes from the rhizosphere of potatoes grown in soybean treated and control soil respectively were examined for antibiotic activity.

The actinomycetes were isolated *) from soil extract agar plates prepared from suitable dilutions of rhizosphere soil by nonselective procedure, all on a plate or plates being taken to permit of a quantitative estimation of the active strains per unit of soil. All isolates were transferred to slants of Conn's asparagine agar and 8 to 12-day cultures at 26°C used for determination of antagonistic action.

For the detection of antibiosis the spot inoculation procedure was used. Standard Petri dishes were poured with 20 ml asparagine agar and held for one to two days at room temperature for evaporation of excess moisture. The actinomycetes were then spot inoculated, 5 cultures to a plate at equidistant points, by means of a 3-mm loop of spore suspension prepared by scraping the agar culture into 10 ml of a freshly prepared, sterile solution of 'Tween 20' diluted 1–5000 with distilled water. After 5 days' incubation at 26°C the

*) We are indebted to Mr. J. W. Rouatt for isolating the cultures used in this study.

plates were flooded with 3 ml of melted agar suspensions of the test organisms, respective series being tested with *Micrococcus pyogenes* var. *aureus*, *Escherichia coli* and *Streptomyces scabies*, the causal agent of potato scab. Suspensions of *S. scabies* were prepared in asparagine agar and those of the others in yeast-beef agar.

Antagonism of the test organism was estimated by measuring the diameter of the zone of inhibition, in the case of *M. pyogenes* and *E. coli* after 24 hours at 37°C; with *S. scabies* the plates were held for 3 days at 26°C. A summary of the results is given in Table I. The chief feature of interest is the increased incidence in the soybean treated soil of actinomycetes with antagonism to *S. scabies*.

TABLE I

Antagonistic properties of actinomycetes in potato rhizosphere		
	Rhizosphere	
	Control	Soybean treatment
Actinomycete strains isolated . . .	103	90
Antibiotic strains		
Against *M. pyogenes* var. *aureus* .	3	9
Per cent	2.9	10.0
Against *E. coli*	2	5
Per cent	1.9	5.5
Against *S. scabies*	1	11
Per cent	0.9	12.2
Millions per gram of soil		
Total actinomycetes	812	211
Antagonistic to *M. pyogenes* . . .	23.5	21.1
,, ,, *E. coli*	15.4	11.6
,, ,, *S. scabies*	7.3	25.7

Mutual relationships of antagonistic actinomycetes. More detailed study was made of the 11 actinomycetes from the rhizosphere of the soybean treated soil which were found to be antagonistic to *S. scabies* out of a total of 90 isolates. Tests were made of the antagonism of each strain to each of the other strains of the active group, as well as that of each of the remaining 79 strains, found to be inactive against *S. scabies*, on each of the active strains.

It was found that only one of the 11 strains active against *S. scabies*, No. S 1, was susceptible to the action of any of the 79 strains, and within this latter group but one strain exhibited any effect. Fur-

hermore, S1, though active against *S. scabies*, was the only one of the active group antagonized in turn by *S. scabies*.

Results from the tests on the mutual antagonisms shown by the 11 active actinomycetes, however, reveal a complicated picture of cross-antagonisms, as shown in Fig. 1. It is noted that all the strains which exert an effect on *S. scabies* are at the same time

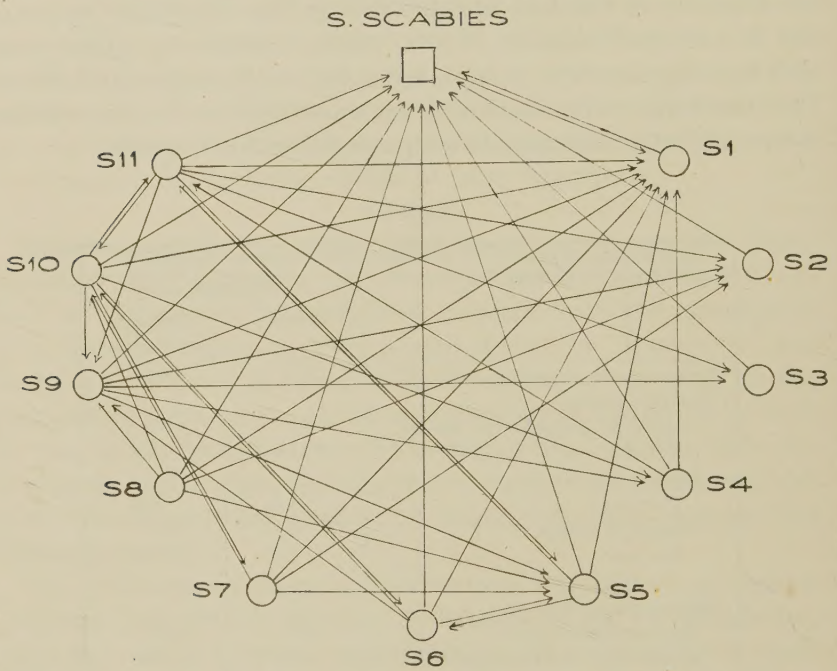

Fig. 1. Mutual antagonisms of 11 strains of actinomycetes showing antibiotic activity against *Streptomyces scabies*.

antagonistic to, or susceptible to the action of, other strains in the group though in widely varying degree. Thus No. S 1 is susceptible to the action of eight of the other strains, No. S 2 to four, while on the other hand No. S 8 is unique in being affected by none of the others though it is itself able to antagonize six of them as well as *S. scabies*.

Discussion. The results shown in Fig. 1 are given to illustrate the multiple inter-relationships which may exist between soil micro-organisms though the example is limited to but one group, the

actinomycetes. The findings suggest in a graphic way possible reasons for failure of an arbitrarily chosen antagonist, which may be active when exposed only to a susceptible organism, e.g. a plant pathogen, to exert an effect in soil where the influence of other antagonists comes into play.

Another important factor which has received scant attention in studies of pathogen-antagonist relationships is the 'rhizosphere effect' — the relation of the plant to pathogen, antagonist and other soil micro-organisms. The growing plant, in addition to causing a pronounced increase in total numbers of micro-organisms in the soil influenced by the root system, has been found to exert a marked effect on the balance between organisms of different morphological groups as well as those of different nutritional requirements [3], [4], [10], [11]. Furthermore, unpublished data from this laboratory indicate that the relative incidence of organisms with antibiotic properties towards a variety of test organisms is different in the rhizosphere from that in control soils. Thus the rhizosphere microflora varies both quantitatively and qualitatively from that of soil more distant from the plant. A given antagonist, therefore, present in or added to the soil finds itself in a changed environment as a rhizosphere is created following germination of the seed, a consideration which may assume great importance in its capacity to thrive and exert its antibiotic action. Thus an antagonistic micro-organism, to be effective, should not only be able to maintain its existence when exposed to the competition and antagonism of the indigenous soil microflora, but be able to exert its antibiotic action when the microbiological balance is shifted and different competitions and antagonisms come into play through the action of the growing plant.

Summary

Attention is called to certain aspects of microbial antagonisms in soil. Tests on the interaction of 90 strains of actinomycetes, isolated by non-selective procedure from the rhizosphere of potatoes grown in scab-infected soil which had been favorably modified by organic amendment, showed that within a group of 11 strains, found to be antagonistic to *Streptomyces scabies*, a wide range of cross-antagonisms existed. The possible importance of this in the study of specific antagonist-pathogen relationships is indicated, as well as that of the 'rhizosphere effect' in modifying the microbiological equilibrium in soil.

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